UV radiation from sunlight

To the Minister of Housing,
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In a letter dated May 19, 1992, (ref. DGM/SVS/14592003) the Minister of Housing, Spatial Planning and the Environment asked me to advise him on the risks for humans and ecosystems resulting from ultraviolet radiation. Special attention was called for the expected increase in risks caused by depletion of the ozone layer.

In order to answer the questions posed in the request for advice, I have installed the 'Risks of UV Radiation' committee on September 17, 1992. The committee laid down its conclusions and calculations into a report that I have subsequently advanced to the Standing Committee on Environmental Factors and Health and the Standing Committee on Radiation Hygiene. Herewith I present you this report.

The committee concludes that human exposure to UV radiation may result in adverse effects: reduction of the immune system, burning of the skin, induction of skin tumors (both carcinomas and melanomas) and the induction of eye disorders. A quantitative relation between the amount of UV radiation received and adverse effects has only been found for sunburn of the skin and for the induction of carcinomas. The committee has calculated the expected increase in the number of carcinomas and the increase in mortality resulting from these tumors. The committee concludes that, even if the emission of ozone depleting substances would be halted immediately, the continuing depletion of the ozone layer in term still will result in several thousands of extra cases of skin carcinoma and several dozens of extra deaths.

With regard to the effects on ecosystems, the committee concludes that present knowledge is incomplete. The committee does signal a potentially threatening development in that it is not unimaginable that the increase in the amount of UV radiation will induce a change in global climate.

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Based on these considerations the commitee makes two suggestions that I would like to stress. Firstly it is desirable that the emission of ozone depleting substances be ceased as soon as possible. This is the only way to limit the effects on ecosystems. Secondly the population needs to be recommended to abstain as much as possible from exposure to UV radiation or sunlight, or to expose itself at least in a sensible way, i.e., not during those hours when the sun is at its highest and to use adequate protective means.

(signed)
Professor Dr L Ginjaar

UV radiation from sunlight

Report of a Committee of the Health Council of the Netherlands

to

the Minister and State Secretary for Housing, Spatial Planning and the Environment

the Minister and State Secretary for Welfare, Health and Cultural Affairs

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Contents

Summary, conclusions and recommendations

Depletion of the ozone layer results in an increase in ultraviolet radiation reaching the surface of the earth. UV radiation has a beneficial effect on the stimulation of the formation of vitamin D by the body, but it also causes various adverse health effects.

The possibility that these adverse effects might increase, motivated the Dutch Minister of Housing, Spatial Planning and the Environment to ask the Health Council of the Netherlands to identify these effects. The Minister wants to be informed about five specific possible consequences of UV irradiation: a decrease in the functioning of the immune system, an increase in the incidence of non-melanoma skin cancer and of melanomas and an increase of the mortality associated with these tumours, the influence on the development of eye disorders and the effects on ecosystems. The Minister also asks to quantify the risk for each of these effects.

In the sections following, a summary is presented of the information contained in the report for each of the effects of UV radiation indicated by the Minister. Finally, his specific questions are answered explicitly.

Effects on the immune system

There are more or less independent regulatory immune system pathways active in the skin. The Langerhans cells located in the epidermis present antigen to T-lymphocytes. This forms the first step of a primary immune response. Keratinocytes in the epidermis can produce certain messenger compounds, the interleukins.

Changes in the immune components in the skin may result in systemic immune effects, since there is continuous recirculation of lymphoid cells from the skin to the lymphoid glands by lymph fluid and blood. Recirculating lymphocytes form the basis of immune surveillance and, as such, are extremely important for the resistance against pathogenic agents and tumour cells.

Immunosuppression by UV radiation

There are a number of parallel mechanisms resulting in immunosuppression by UV radiation.

UV irradiation leads to a decrease in the number of Langerhans cells in the skin and to a change in their antigen presentation. This causes a local decrease in resistance that is intensified by the induction of T-lymphocytes with suppressor activity. There is also a systemic influence, since the altered Langerhans cells migrate to the lymphoid glands and can change the pattern of recirculation of the T-lymphocytes. UV radiation also induces keratinocytes to produce various interleukins. These can influence the immune system both locally and systemically.

UV radiation-induced immunosuppression might also be mediated by urocanic acid, a substance formed in the epidermis. *Trans*-urocanic acid can be modified into the *cis*-isomer by UV radiation. *Cis*-urocanic acid has been shown to suppress certain hypersensitivity reactions in the mouse.

Finally, it has been demonstrated recently that UV-induced DNA damage can also play a role in decreasing the immune response. However, the mechanism is not yet clear.

Resistance against tumours

In the mouse, immunosuppression results in failure of the immune system to destroy cells with antigenic properties (for instance, tumour cells). It seems likely that in humans also the influence of UV radiation on the immune system reduces resistance against tumours. The increased skin tumour incidence that is associated with exposure to sunlight could be due in part to UV-induced immunosuppression.

There is an increased risk of skin tumours associated with exposure to UV radiation in individuals that already have a suppressed immune system, for instance in patients who have had an organ transplant.

Resistance against infections

In general, it can be expected that a decreased function of the immune system will affect the resistance against infections. This hypothesis is supported by studies on patients suffering from infection with *Mycobacterium leprae*, the cause of leprosy. Exposure to UV radiation might augment the existing immunosuppression in AIDS patients that are infected with HIV. However, epidemiological studies in this field have not been performed.

Exposure to UV radiation can lead to the activation of latent viruses. Activation of virus replication has been found in HIV-positive individuals. This is, however, not necessarily mediated by the immune system, it can also be a direct effect of UV radiation on the virus.

Another possible effect of UV radiation may be a reduction in the efficiency of vaccination, but there is no information available on this matter.

Protection against photo-immunological effects

Skin pigment does not offer protection against UV-induced immunosuppression. Most chemical sunscreens also do not offer sufficient protection. The SPF, a factor that indicates the degree of protection against sunburn, does not indicate protection against immunosuppression.

Therapeutic application of UV radiation

In a few cases suppression of the immune system by UV radiation can be beneficial. This is exploited in the treatment of certain skin diseases, for instance eczema and *psoriasis vulgaris*.

Effects on the skin

The skin is the organ which has the highest exposure to UV radiation. For this reason, the cellular effects of UV radiation have been investigated primarily in skin cells *in vitro* or *in vivo*. These studies have demonstrated that UV radiation is capable of damaging many molecules and structures in the cell. This may result in changes in cellular functions. DNA damage can lead to the development of a tumour. Mutations on UV-sensitive sites in oncogenes and tumour suppressor genes have been demonstrated in animal and in human cells.

The development of several types of skin tumours seems to be related to exposure to UV radiation: melanomas, originating from the pigment-producing skin cells, the melanocytes, and carcinomas or non-melanoma skin cancers that originate from the keratinocytes, the horn-producing cells of the epidermis.

Non-melanoma skin cancers

The most prominent non-melanoma skin cancers are the basal cell carcinomas and the squamous cell carcinomas. In animal experiments, a causal relationship with exposure of the skin to UV radiation was only demonstrated for the development of squamous cell carcinomas. It has been near impossible to experimentally induce the other type of skin tumour with UV radiation. For basal cell carcinomas the presumed relationship with exposure to UV radiation is based primarily on epidemiological data and the presence of UV-related changes in DNA of tumour cells.

Relationship between exposure to sunlight and the incidence of non-melanoma skin cancer

Squamous cell carcinomas and basal cell carcinomas occur almost exclusively on sun-lit parts of the skin, especially in the caucasian population. People extensively exposed to UV radiation and with a skin that burns easily and hardly tans are most at risk.

A number of factors can be identified that influence the risk of developing a carcinoma. Studies on immigrants in Australia have shown that exposure during childhood and adolescence (before the age of 20 years) constitutes a larger risk factor than exposure at later ages. The reason for this is twofold. First, the time available for carcinogenesis is longer with childhood exposure and for most people childhood exposure dominates the life-time exposure. Second, children are likely to have relatively more proliferating skin cells than do adults. Since the mutagenic effect of UV radiation is considerably greater in dividing than in resting cells, the risk of a cell contracting a mutation such that its proliferation is disturbed is correspondingly greater.

It has already been mentioned that suppression of the immune system increases the risk of skin cancer. Kidney transplant patients treated with immunosuppressive drugs for the rest of their lives have an approximately 500 times higher risk of the development of carcinomas. Exposure to sunlight before the age of 30 years is also highly associated with the development of carcinomas in these individuals.

An increased incidence of carcinomas was also observed in other persons with a suppressed immune system, for instance AIDS patients. The risk of metastases and mortality is also greater in these people.

Finally, (immuno)genetic factors possibly play a role in the development of carcinomas. It is important to examine whether such factors in general predispose for the development of carcinomas.

The committee believes that the relation between accumulated exposure to sunlight and squamous cell carcinoma has been demonstrated convincingly. It is clear that there is also a relation between exposure to sunlight and the development of basal cell carcinoma, but this relation is not as straightforward as that for squamous cell carcinoma. Judging from the localisation of basal cell carcinomas on the body, most of these tumours (80 - 95%) are induced by sunlight.

Dose-effect relationship for non-melanocytic skin cancer

Epidemiological and experimental studies have shown that the incidence of skin tumours is a power function of UV dose as well as of age. The values of the exponent for the UV dose in humans, approximately 2.5 for squamous cell carcinomas and 1.5 for basal cell carcinomas, indicate that the incidence is highly responsive to alterations in the UV dose. In practice this means that an increase in dose will lead to tumours becoming manifest at an earlier age and to an increased number of tumours.

The values of the exponent for the UV dose for squamous cell carcinomas in humans are lower than those in mice. This indicates that, in humans, the induction of these tumours depends less on the UV dose than in mice. The values of the age-dependence exponent in humans are comparable to the ones in mice.

The corresponding values for basal cell carcinomas are lower. Since the exponents are probably proportional to the number of steps in the process of carcinogenesis that are related to the UV dose and to age, this might indicate that more steps are required for the induction of a squamous cell carcinoma than for the induction of a basal cell carcinoma.

Quantitative data on incidence and mortality are given in the answers to the questions of the Minister.

Melanomas

The literature shows that for melanomas the relationship to sunlight exposure is much less unambiguous than for carcinomas. Considerable differences exist between the various types of melanomas and the exposure pattern also seems to have a significant influence.

Some melanomas are found frequently in parts of the skin that are not regularly exposed to sunlight but that had to deal with a large dose of sunlight within a short period of time, for instance during a summer holiday. On the basis of these observations, the intermittent exposure hypothesis was conceived. This hypothesis assumes that for these types of melanoma the dose rate is of greater importance than the total dose. With other types of melanoma, the total dose does seem to be the most important factor. There are also indications that exposure during childhood is of relatively greater importance for the induction of certain melanoma types than exposure later in life.

The principal types of melanoma are lentigo malignant melanoma, which almost exclusively occurs on the face or the lower arms, especially in long-term exposed people; superficially spreading melanoma, the most frequently diagnosed skin melanoma; nodular melanoma and acrolentiginous melanoma. The latter type is, by definition, localised on the acra: the palms, the soles, under the nails and also on the lips. It is the most frequently diagnosed type in coloured races.

Incidence and mortality

The incidence of skin melanomas has increased in the caucasian population by a factor 2 or 3 during the past 30 years. This increase is largely due to tumours on skin parts with a low sunlight exposure. No increase was observed during this period in the coloured population, in which the melanoma incidence already is significantly lower. Skin pigmentation therefore seems to protect against melanoma induction.

The incidence data for the Netherlands seem to indicate a doubling in melanoma incidence between the years 1975 and 1983, but interpretation of these data is complicated by incomplete registration. It is clear, however, that the incidence of melanomas in the Netherlands is relatively high: approximately 130 cases per million persons per year.

Mortality from melanomas has also increased. In general, this increase is less than the increase in incidence. Improved diagnostic procedures that result in earlier detection may be the reason. In the Netherlands, mortality quadrupled between 1950 and 1988. A possibly gradually improving registration of melanoma as a cause of death may have contributed to this increase. Registered mortality due to melanomas was relatively low in 1950 (approximately 4 per million per year), but in 1988 it matched that in other industrialised countries (approximately 16 per million per year).

Etiological factors

When epidemiological data are used to determine etiological factors it should be kept in mind that the various types of melanoma probably develop in different ways. It is

therefore important to stratify the incidence data for the different clinical types of melanoma.

On the basis of epidemiological data, lentigo malignant melanoma seems to be related to cumulative sunlight exposure and superficial spreading melanoma and nodular melanoma to short excessive exposure. It might have been possible that in some studies exposure to sunlight was better remembered by patients than by controls (recall bias).

There is also a difference in incidence of melanomas in Australia between the original caucasian population and immigrants. The relative risk of developing superficial spreading melanoma is significantly higher for those people who moved to Australia before the age of 15 years compared to those who moved later in life.

The committee thinks that the hypothesis that childhood exposure has a major influence on the development of melanoma is based on more solid grounds than the hypothesis of intermittent exposure. The latter strongly depends on the recollection of the interviewed individuals of exposures in the past. This is not the case in the immigrant studies.

Other melanoma risk factors

Factors other than sunlight exposure also play a role in de development of melanoma. One of the most important is a genetic defect: the FAMMM (familiar atypical multiple mole melanoma) syndrome. Patients with this defect usually present with a large number of moles and -also as a result of this- they have a greatly increased risk of developing melanoma. The gene responsible for the FAMMM syndrome, probably a tumour suppressor gene, was recently localised. Isolation of this gene may lead to a breakthrough regarding the development of melanoma.

The effect of UV radiation on metabolism of pigment may also be important in melanogenesis. The balance between the two types of melanin, pheomelanin and eumelanin, is decisive. People with relatively high levels of pheomelanin and low levels of eumelanin, such as fair-skinned persons, are at an increased risk of developing melanomas. Pheomelanin can be damaging once it is activated by UV radiation. The exact mechanism involved is still unknown.

In the answers to the questions of the Minister the committee indicates why it feels that it is not possible to calculate the risk of contracting a melanoma as a result of exposure to UV radiation.

Effects on the eyes

Two lesions of the eyes that threaten vision are possibly related to damage caused by UV radiation: senile cataract and senile macular degeneration. Their names indicate that they are primarily associated with advancing age. The reason might be an endogenous ageing process or a relation with long-term effects of toxic factors, e.g. UV radiation.

In considering the effects of UV radiation on the eyes the committee refers only indirectly to traumatic damage such as welders' eyes and snow blindness, because the development of these lesions is primarily related to behaviour.

Lesions of the retina and cornea

Senile macular degeneration is the most important cause of blindness world-wide and is not yet treatable. The prevalence of this lesion strongly increases with age: from 4% to 12% between the seventh and eighth decade of life to 30% in the ninth decade. There are virtually no reasons to assume that UV radiation plays an important role in its etiology. No or almost no UV radiation reaches the retina in adults. Up to the age of about 20 years, some lesions are found, but these are only traumatic retinal lesions.

The two most frequently occurring corneal lesions are pterygium and pinguecula. There are almost no data concerning the prevalence of these lesions. It is clear, however, that their prevalence is significantly less than that of senile cataract and macular degeneration. Exposure to UV radiation is considered to be a causal factor, but the committee feels that there are few arguments indicating that it is an important factor. The influence of wind and salt (at sea), and dryness and sand (in desert areas) could also play a role in the development of these lesions.

Lesions of the lens

Lenticular lesions are more frequent than retinal ones. Therefore it is assumed that changes in the lens are the most important cause of decline of vision during ageing.

Cataract is the most frequently occurring eye disease. The increase in its prevalence occurs at a much lower age than in the case of macular degeneration. Approximately 16% of the population of the Netherlands over 50 years of age suffers from senile cataract. At high ages (75 - 80 years) this can increase up to about 46%.

Without treatment, cataract inevitably leads to blindness. Effective treatment is possible by surgical removal of the diseased lens and implantation of a new (artificial) lens.

UV radiation as a cause of cataract

When assessing the data on the relation between exposure to sunlight or UV radiation and the development of cataract, the committee faced two 'schools of thought' in the scientific community. Supporters of the first think that UV is an important cause of cataract. Supporters of the second feel that this is not the case, but that other factors such as the nutritional situation and hygienic circumstances are crucial. At the suggestion of the committee the Health Council of the Netherlands organised an international workshop where 14 experts, experimentalists, clinicians and epidemiologists, representatives of both 'schools', debated this subject. The workshop, entitled: 'The UV Scenario for Senile Cataract: Fact or Fiction ?' was held in Rotterdam, January 31st and February 1st, 1994. The data presented and the discussions held have supported the committee in drawing her conclusions about the relationship between exposure to sunlight and the development of cataract.

Experimental studies unquestionably demonstrate that UV radiation can have a direct effect on the lens and can induce lesions in the lens cortex and nucleus.

The epidemiological studies into the relation between lenticular lesions and exposure to sunlight are not always unequivocal. It is often difficult to determine consistence between the different studies, especially since the definitions used for cataract may differ.

The study that best complies with the criteria for sound epidemiological research shows that there is a positive relation between the occurrence of opacities in the anterior cortex (the outer layer of the lens) and sunlight exposure. This is considered as an indication that also cortical cataract is related to exposure to sunlight. Indications have been found for a weakly positive relation for posterior subcapsular cataract (located in the posterior cortex of the lens), while for nuclear cataract (located in the central part of the lens) such a relation seems to be completely absent.

By themselves, other studies do not offer firm indications for a relation between exposure to sunlight and the development of any form of cataract -some because they were restricted in size, others as a result of a sometimes inevitably suboptimal design- but they can be considered as support for the above mentioned studies.

Summarising conclusions as well as some remarks on quantification are given in the answer to the specific question of the Minister.

Effects on ecosystems

The committee has made an inventory of available knowledge concerning the effects of UV radiation on ecosystems, making a distinction between different levels of organisation.

Effects on abiotic structures

UV radiation can cause the break-down or alteration of the availability of water-dissolved substances that are important for growth and functioning of cells, for instance vitamin B12, amino acids and trace elements, e.g. iron. UV radiation can also promote the formation of carbonyl sulfide, a volatile sulfuric compound that is formed in the top layer of sea-water with a high content of organic matter. In the stratosphere carbonyl sulfide, which is very persistent in the atmosphere, induces the formation of haze. This can function as a negative feed-back mechanism for the amount of UV radiation reaching the earth's surface.

A positive effect is that also the concentration of xenobiotic compounds, e.g. pesticides, can be reduced by UV radiation. On the one hand the absorption of such compounds to organo-metal complexes can be reduced, on the other hand the direct photochemical breakdown can be increased.

No data on dose-effect relations are available for any of these processes.

Effects at the level of the cell and individual

In animal and plant cells, UV radiation can directly or indirectly cause alterations in DNA. This may result in the disturbance of cellular processes. Additionally, direct damage can be inflicted on the photosystem in plant cells, which reduces photosynthesis. These alterations in functions and structures of the cell can result in changes in functions and structures of organisms.

In plants, UV radiation can stimulate the formation of UV-B-absorbing compounds. It can also reduce the production of compounds that act as defense against herbivores. This can alter the herbivores' feeding pattern and, as a result, the composition of the plant population. Changes in the molecules in the cell walls can also change the digestion of plant material.

Only incidental data are available on these processes, insufficient to determine dose-response relations. In some cases an action spectrum has been constructed. At this time it is not possible to determine a quantitative relation between UV irradiation and the effects mentioned.

Effects at the level of the population

The sensitivity of organisms is determined first by their genetic composition. In the reaction of a population, the genetic variation also matters. Many plant species investigated contain UV-sensitive and UV-resistant genotypes. Therefore populations can react to an increase in the UV dose by shifts in the dominance of genotypes.

The sensitivity of a population is further determined by both present and past selection resulting from exposure to UV radiation.

The position of the species in the ecosystem is of importance for the extent of natural exposure to UV radiation. Plants and animals living under the closed crown roof of a forest are hardly exposed to UV radiation. The same holds for organisms with a short life-cycle in spring or for nocturnal organisms. In aquatic systems there are large differences in exposure as a result of variations in the penetration of UV radiation in water and in the depth at which organisms live.

Effects at the level of the ecosystem

Only when the position of the species or populations in an ecosystem is known and information is available about their daily or seasonal activity and about the effects of UV radiation, it might be possible to describe the consequences for ecosystems of an increase of the level of UV radiation.

Some algae and plant species are particularly sensitive to UV radiation. Therefore, shifts in species composition might occur in ecosystems that are exposed to increased UV radiation. The possible changes in terrestrial ecosystems resulting from alterations in the feeding pattern of plant-eaters were already mentioned. Hardly anything is known about these 'indirect' effects on ecosystems. It is therefore not possible to quantify a relation between an increase in UV radiation level and changes in ecosystems.

Consequences of human behaviour

Socio-economic factors influencing the way man interacts with nature may promote UV-induced damage to terrestrial ecosystems. The vegetation replacing cut-down rain forests might be less adapted to increased UV radiation than the original one, possibly leading to indirect environmental damage, such as increased erosion. It is also possible that sensitive links in ecosystems disappear as a result of synergistic effects between discharged toxic chemicals (for instance PACs) and increased levels of UV radiation.

Climatological circumstances

Cloud cover is one of the factors that determines the amount of UV radiation reaching the earth's surface anywhere on earth. Changes in cloud cover therefore have direct consequences for the amount of UV radiation in the biosphere.

It is conceivable, but far from demonstrated, that changes in ecosystems resulting from an increase in UV level may lead to changes in the world climate through alterations in cycles of natural elements and in the discharge of certain (greenhouse) gases into the atmosphere. The committee thinks that this is one of the most threatening scenarios. Also in this case, however, insufficient data are available to allow any quantification.

Preventive measures

UV radiation constitutes a natural risk. The effects to humans as well as to (components of) ecosystems of an increase in UV exposure resulting from ozone depletion constitute added risks. One of the elements of the environmental policy of the government is to reduce added risks. In order to accomplish this, the government uses measures aimed at both the source and the effect. The committee offers several suggestions for such measures.

Source-directed measures

In the case of UV irradiation, reducing the extra amount of UV irradiation resulting from depletion of the ozone layer is the only feasible source-directed measure. The only way this can be accomplished is to stop the emission of substances affecting the ozone layer.

Limitation of exposure

Informing the public about the dangers of exposure to UV radiation can be effective. Major campaigns in Australia informing about the risks of developing skin cancer have resulted in a general reduction of exposure of the public to sunlight.

From the point of view of reducing risks it would be best to avoid sun-bathing completely. In order to minimise the risk of harmful effects of UV radiation, it is important to avoid sunburn, especially in children. During the summer months one should stay away from direct sunlight between 11.00 and 15.00. When engaging in outdoor activities (sports, recreation), it should be advised to use sunscreens. Although

sunscreens adequately protect against sunburn and some protection against UV-induced DNA damage has also been observed, they do not always offer protection against UV-induced immunosuppression and it is uncertain whether they can prevent the development of skin cancer. Therefore, it is not advisable to use sunscreens to allow a longer stay in sunlight than would be deemed possible without this protection.

The consequences of UV-induced immunosuppression are not yet fully known. Nevertheless, the committee would like to recommend, even at this early stage, that people who are at risk for immunosuppression abstain from excessive exposure to sunlight.

Welders' eyes and snow blindness, the traumatic eye lesions caused by UV radiation, can be prevented by taking sufficient protective measures: wearing welding goggles or snowglasses, respectively. Snowglasses should have shielding on the bottom and sides as well, since a major portion of UV exposure in snow-covered areas results from reflected UV radiation (fresh snow can reflect more than 80% of incident radiation).

In order to reduce the influence of UV radiation on the development of cataract or any other chronic eye disorder, it is recommended to protect the eyes as much as possible from UV radiation. The amount of direct radiation can be reduced significantly by wearing a hat with a brim or a cap. Sunglasses can also be effective if they do not transmit UV radiation. If they do, wearing such glasses may have an adverse effect: the reduction of the amount of visible light will cause the pupil to dilate and hence more UV radiation can reach the lens. Even sunglasses that block all UV still cannot fully protect the lens from this radiation. Significant amounts of UV radiation can reach the eye from the bottom and sides as a result of reflection. This is especially the case near or at reflecting surfaces such as water and light sand. In such cases the wearing of sunglasses that offer adequate shielding on the bottom and on the sides, as do snowgoggles, is recommended.

In view of the experience in Australia, the committee expects that, given adequate education and information, it should be possible to bring about a change in behaviour of the public. In that way the effects on humans of an increase in the amount of UV radiation resulting from depletion of the ozone layer might be more than compensated.

Answers to the questions of the Minister

Question 1: Are there new scientific developments after the 1986 advisory report that more clearly relate exposure to UV radiation and effects on the human immune system? Is it possible to translate such effects, for instance immunosuppression and the related increase in, e.g., infections, if they can be demonstrated,

into dose-effect relations for diseases or mortality? If such dose-effect relations can be quantified, what is the chance for mortality and/or incidence of disease?

Animal experiments show that UV radiation has effects on the immune system. These effects may result in a reduction of the defense against infections and against the growth and development of tumours. Effects on immunological parameters in humans are also known.

Although the immune systems in humans and animals are grossly comparable, extrapolation of experimental data to humans is difficult. Therefore it is possible that effects that were observed in animals can lead to overestimation, but also to underestimation of the seriousness of effects in humans. This is true for infectious diseases as well as for the growth of tumours.

An increased exposure to UV radiation resulting from depletion of the ozone layer is likely to influence the prevalence of infections and possibly also that of tumours through effects on the immune system. The available data are still insufficient to make quantitative estimates of the risk of health effects.

Question 2: Many model calculations make a connection between depletion of the ozone layer and a certain increase in UV radiation in the biosphere, resulting in an increase in the incidence of non-melanoma skin cancer. Have new views been developed of the value of the risk factor that relates this cancer incidence and exposure to UV radiation? The same question can be asked with respect to the quantitative relation between mortality resulting from skin cancer and the incidence of cancer resulting from exposure to UV radiation.

After the report of the Health Council of the Netherlands was issued in 1986 more data have become available concerning the relation between UV irradiation and skin cancer. This has resulted in a reduction of the uncertainties connected to the risk estimates.

According to the present estimates, one percent increase in the amount of UV radiation leads to 2.5% increase in squamous cell carcinoma incidence and 1.5% in basal cell carcinoma incidence. Each percent ozone layer depletion leads to an estimated increase in the incidence of squamous cell carcinomas by $3.1 \pm 0.9\%$, and of basal cell carcinomas by $1.9 \pm 0.5\%$.

On the basis on model calculations, the increase in the incidence of carcinomas expected as a result of ozone layer depletion will continue to increase up to the year 2040, even with the most favourable CFC production scenario. Approximately 190 additional tumours (squamous and basal cell carcinomas combined) per million people per year are expected at that time in the Netherlands. As a result of the long latency period of the induction of skin carcinomas, the increase in tumour incidence lags several

decades behind the increase in the amount of UV radiation. After 2040 the increase in incidence may slowly subside.

The committee estimates that a sustained 10% decrease of stratospheric ozone could in term result in 55 ± 20 additional cases of squamous cell carcinoma per million people per year and in 180 ± 75 additional cases of basal cell carcinoma per million people per year, assuming that behaviour is not changed. For the Netherlands, assuming a population size of 15 million, this amounts to approximately 825 additional cases of squamous cell carcinoma and 2700 additional cases of basal cell carcinoma per year.

With skin carcinomas, the incidence is much higher than the mortality. Squamous cell carcinoma mortality is approximately 3% of the incidence and for basal cell carcinoma it is approximately 0.3%.

Using these values and the numbers for the incidence mentioned above, a sustained 10% reduction of stratospheric ozone will in term result in an estimated number of additional deaths resulting from squamous cell carcinoma of 1.7 ± 0.6 per million people per year and from basal cell carcinoma of 0.5 ± 0.2 per million per year. For the Netherlands, assuming a population size of 15 million, this amounts to approximately 25 additional deaths resulting from squamous cell carcinomas and 8 additional deaths resulting from basal cell carcinomas. As a result of the mentioned long latency period, this will primarily concern older people.

Question 3: Until recently it was not clear whether UV radiation may also (partly) be responsible for the induction or stimulation of the development of melanomas. Is there at this time, based on recent scientific developments, more certainty about the relation between UV radiation and the melanoma incidence, especially about the possible role of UV radiation as a co-factor? Can such relations be quantified? Are there any new developments that are important with respect to the determination of the mortality risk associated with melanomas?

The relation with exposure to sunlight is considerably less clear for the development of melanomas than it is for carcinomas. Large differences exist between the different types of melanoma and also the pattern of exposure seems to be very important. The incidence of superficial spreading and nodular melanomas is increased in parts of the skin that are not regularly exposed to sunlight, but that have to deal with a large dose within a short period of time, for instance during summer holidays. These observations led to the development of the hypothesis of intermittent exposure, which assumes that the dose rate is more important than the total dose in the development of these types of tumours. The total dose seems to be the most important factor for lentigo maligna melanoma. There are also indications that exposure during early childhood is of

relatively greater importance for the development of certain types of melanoma than exposure later in life.

The committee feels that the hypothesis that sunlight exposure during childhood has a major influence on melanoma risk is based on more solid grounds than the intermittent exposure hypothesis.

One of the major problems in quantifying the melanoma risk is that no adequate experimental data are available about the wavelength dependence of tumour induction in humans.

Therefore the committee considers it is improper and unwise to perform calculations or make predictions on the effects of an increase in the amount of UV radiation on the incidence of melanomas or on the mortality associated with these tumours.

Question 4: Have sufficient indications become available that show that more eye disorders than previously assumed can be related to exposure to UV radiation? Is it possible to give so-called risk numbers based on existing and new data on UV exposure-induced eye diseases?

Experimental studies indicate sufficiently clear that UV radiation can cause lens opacities that may lead to cataract. Epidemiological studies show a positive relation between exposure to sunlight and the prevalence of opacities in the anterior cortex (the outer layer of the lens). This is considered to be an indication that cortical cataract is related to sunlight exposure. Other data indicate a weakly positive relation between exposure to sunlight and the development of posterior subcapsular cataract (located in the posterior cortex of the lens). No relation seems to be present for nuclear cataract (located in the central part of the lens).

The high prevalence of cataract in tropical areas is likely related to the often poor nutrition and poor hygienic circumstances. These factors are sometimes mentioned as much more important causal factors than exposure to UV radiation.

At present it is not possible to determine the importance of UV radiation in the development of cataract relative to that of other factors that possibly play a role. The contribution of UV radiation strongly depends on different external factors, such as behaviour. It is also not possible to quantify the effect of an increase in UV radiation resulting from ozone layer depletion on the prevalence of cataract.

Question 5: In the past, research related to the effects of UV radiation was primarily aimed at effects in humans. Increasingly, questions are asked about the possible negative effects of UV radiation on ecosystems. Has scientific knowledge already been developed in such a way that possible negative effects on ecosystems, for instance aquatic ecosystems, can be quantified? Does the system on handling effects in

ecosystems, that has been presented in the publication, 'Dealing with risks', offer any help in performing such quantifications?

The committee has made an inventory of the knowledge available concerning the influence of UV radiation on ecosystems. It has made a distinction between different levels of organisation: abiotic structures, cells, individuals, populations and ecosystems.

At every level, only incidental data are available, insufficient to determine dose-effect relations. In one or two cases an action spectrum has been constructed. It is not possible at the present time to quantify any relation between UV irradiation and the known effects.

It is conceivable, but far from demonstrated, that changes in ecosystems resulting from increased UV irradiation may lead to changes in the world climate through alterations in cycles of natural elements and in the discharge of certain (greenhouse) gases to the atmosphere. The committee thinks that this is one of the most threatening scenarios, but at the same time stresses that there are no indications at this time that a catastrophy is at hand. No data are available to allow any form of quantification.

Finally, the committee feels that the system for determining the risks for ecosystems as described in the publication, 'Dealing with risks', cannot be applied to the effects of UV radiation. The main reason is that UV radiation not only has an influence on individuals and species, but also on abiotic elements of ecosystems and on several element-cycles.

Chapter 1

Introduction

1.1 Motivation for the request for advice

Industrial developments of the past century have led to considerable economic progress and prosperity for large parts of the world population. On the other hand the emission of numerous substances in the air, soil and water accompanying industrial processes has led to significant environmental problems. Initially these negative consequences of increasing prosperity were local and regional. Some environmental problems have, however, assumed world-wide proportions. As a result of this, global climate changes are not unlikely. In recent years a number of measures and rules have led to improvements in the quality of the environment at the local and regional level. Dealing with global problems is, however, hampered by considerable difficulties.

One of the most important global problems is depletion of the stratospheric ozone layer. This layer protects the earth against the harmful ultraviolet (UV) radiation from the sun. It has been shown that the reduction in the amount of ozone in the stratosphere is the result of human actions.

In the fifties, various chlorofluorocarbons (CFCs) had been developed, to be used as, e.g., refrigerants in refrigerators and air conditioners and as inert propellants in sprays. These CFCs have mostly ended up in the atmosphere and have gradually penetrated into the stratosphere. In 1974 it was shown that CFC molecules in the stratosphere are broken down by high-energy UV radiation and that the released chlorine atoms subsequently act as catalysts in the breakdown of ozone molecules. The consequence of these processes is the threatening increase in the amount of UV radiation

from sunlight that reaches the surface of the earth. This UV increase is predominantly in the range of wavelengths that cause most biological effects, and is, therefore, a potential danger for human and environmental health. In reaction to this discovery, several countries have restricted the application and use of CFCs. The government of the Netherlands has set limits, especially with regard to the use of CFCs in cans with sprays. In spite of these efforts large quantities of these substances still end up in the atmosphere.

In 1984 English researchers reported that each year in the (austral) spring a considerable reduction in the thickness of the stratospheric ozone layer occurs over Antarctica. This results in an increased amount of UV radiation reaching the earth's surface. Since these initial observations, continuous monitoring has shown that the so-called 'ozone hole' is still expanding. Ozone depletion, while less, has also been shown in other seasons and at other latitudes. Furthermore it was shown that CFCs are not the only compounds with this effect on the ozone layer. Halons, used in e.g. fire extinguishers, have also been shown to have this effect.

The observation of an actual ozone layer depletion has led to international discussions aimed at reduction of the emission of ozone-depleting substances. In 1985 an international treaty, the Vienna Convention for the Protection of the Ozone Layer, was negotiated. At subsequent meetings in Montreal, London and Copenhagen increasingly far-reaching measures have been agreed, in order to limit the production and use of ozone-depleting substances. The program set up by the Dutch government goes beyond these measures (TK92a, TK92b, TK93). However, even with the most rigorous measures, i.e. immediate, full stop of production and use of these substances, there will remain a continuous flow to the stratosphere until well into the next century. It is therefore to be expected that depletion of the ozone layer will continue for some decades. Therefore the risk of an increase in the amount of harmful UV radiation reaching the earth's surface will also remain. It should be mentioned that, on a global scale, there are major differences in the 'natural' UV load and its possible increase.

UV radiation has a beneficial effect in that it stimulates the synthesis of vitamin D in skin (GR86). A 15-minute exposure of the skin of the head and hands to the afternoon sun suffices for this effect. However, UV radiation is harmful to health in several ways. It can result in 'burning' of the skin and stimulate the development of certain types of skin cancer. It can also adversely influence the immune system and damage the eyes. Moreover, UV radiation can be harmful to plants and animals. An increase in the amount of UV radiation may disturb the natural balance of both aquatic and terrestrial ecosystems. This may indirectly affect human health and well-being.

Various bodies have made public their concern over the results of ozone layer depletion through reports and recommendations for measures to be taken. A committee of the United Nations Environmental Program (UNEP) issued a report on this matter

in 1989 (UNEP89). UNEP concludes in a follow-up report in 1991 that further measures are necessary to allow recovery of the ozone layer, at least in the long run (UNEP91). The threat of an increase of UV radiation is still fully present. Together with this, new harmful effects of UV radiation are being recognised.

The possibility that the negative effects on the well-being of humans, plants and animals might increase, led the Dutch Minister of Housing, Spatial Planning and Environment to ask the Health Council to identify these effects. One of the policies of the Ministry is to limit the risks for the population and for ecosystems in the Netherlands that are related to exposure to different environmental factors, such as substances and radiation. Quantification of the risks attendant to exposure to UV radiation can contribute to this goal. In the present report, a committee of the Health Council that was installed for the purpose attempts to indicate the extent to which an increase of the 'natural' amount of UV radiation influences the occurrence of negative effects and in how far this can be expressed as a risk number.

1.2 The approach to risk in environmental policy

In the National Environmental Policy Plan the government chose an aim at sustainable development as the basis of its environmental policy. One of the requirements of such an approach is prevention of adverse effects to humans, animals and plants, environmental functions and materials. In the document, 'Dealing with risks', the government indicated what it considers to be adverse effects and what approach it follows to limit or prevent these effects (TK89). The so-called source-directed approach aims at reduction of the emission of noxious agents, while the effect-directed approach seeks to control and limit negative environmental effects that are the result of human activities. Quantification of the risks attached to agents can be a useful tool in these approaches. In the case of humans, protection of the individual is the starting point and risk for the individual is the criterion. In the case of plants and animals the unit to be protected is the population, and for ecosystems it is the system as such. Compared with substances, radiation is a special agent, because there is often a relatively high natural background. This also applies for ultraviolet radiation: were the ozone layer completely unaltered, a considerable amount of UV radiation would still reach the earth's surface.

With respect to UV radiation the government is interested in the amount of extra radiation reaching the earth's surface as a result of human-induced ozone depletion. Environmental policy is focused on limitation of the extra risk that is added to the 'natural' risk (TK89).

For humans, the threshold value of the individual risk of death due to, for instance, an increase in the amount of ionising radiation above background, is set at 10^{-6} per year. This is the 'maximum permissible risk'. The risk policy for ecosystems is

directed at another level, species and systems. For substances with a threshold value, the maximum permissible risk for ecosystems is set at the level that offers protection to the species in a system. The limiting value in the determination of this level is protection of 95% of the species. While this is possible in principle, these limits have not yet been declared to apply to UV radiation.

1.3 The request for advice

In 1986 the Health Council issued the report titled 'UV radiation' (GR86). This report dealt specifically with the influence of UV radiation on the development of non-melanocytic skin cancer. The committee then at work indicated in the report that further effects of UV radiation might be important for human well-being. It specifically mentioned a possible influence on the development of melanomas and on reduction of immune system functioning. While insufficient scientific data were available at the time to allow meaningful conclusions with regard to these points, current scientific progress now offers clearer indications.

Also it has become clear in recent years that an increase in the amount of UV radiation is not only a public health problem, but also has effects on the structure and functioning of ecosystems. Scenarios can be conceived of in which disturbance of the natural balance at the bottom of the food-pyramid has far-reaching consequences for the food supply at the top, e.g. for humans. It is also conceivable that disturbances may occur in element cycles that might contribute to changes in global climate.

The Minister's request for advice, the full text of which is given in annex A, focuses on five different effects of UV radiation: reduced functioning of the immune system, increase in the incidence of non-melanocytic skin cancer, increase in the incidence of melanomas, development of eye disorders and the effect on ecosystems. In the present report the committee presents an overview of the scientific data regarding these items, as well as background information that is necessary for the interpretation of these data.

1.4 The committee and its procedures

On September 17, 1992, the President of the Health Council installed the 'Risks of UV radiation' committee - referred to here as 'the committee' - that was charged with answering the questions posed by the Minister. The committee members are listed in annex B.

At its installation meeting the committee decided to perform its activities concerned with drafting of this report as two working groups. Annex B contains the composition of these groups. The 'Effects on humans' working group met 14 times and the

'Effects on ecosystems' group met 4 times. After these working groups had concluded their activities the committee in full met once to discuss its complete report.

In order to correctly interpret the sometimes contradictory data on the effects of UV radiation on the eyes, specifically on the lens, the committee deemed it necessary to consult external experts. As proposed by the committee, the Health Council organised an international workshop on January 31 and February 1, 1994. At this meeting, entitled 'The UV Scenario for Senile Cataract: Fact or Fiction ?' fourteen experimental, clinical and epidemiological experts gave their opinion on the effects of UV radiation on the lens. The committee was greatly supported in the formulation of its conclusions by the presentations and the discussions at this workshop. A report on the workshop is presented in annex C.

1.5 Structure of this report

The present report starts with several introductory chapters. In chapter 2 the committee indicates the extent of exposure to UV radiation, and the magnitude and the causes of changes in the amount of UV radiation at the earth's surface. In chapter 3 the committee discusses factors that are of importance in the correct interpretation of epidemiological studies. This is relevant for the effects of UV radiation on skin and eyes. In chapter 4 the committee presents an overview of the effects of UV radiation on cells and tissues. In subsequent chapters it describes the effects on the human body, gives the best possible quantifications and risk calculations of these effects and makes suggestions for further research if the data available are insufficient to allow reliable quantification. The committee chose an approach according to which 'targets' of UV radiation are dealt with separately. The fifth chapter deals with the effects of UV radiation on the immune system. These effects are important since they might have indirect consequences for the reaction of skin and eyes to UV radiation. Therefore the committee discusses first the effects on the immune system. Certain aspects of the influence of UV radiation on the immune system are referred to again when relevant in the chapters on effects on skin (chapter 6) and effects on the eyes (chapter 7). The next two chapters of the report deal with the influence of UV radiation on ecosystems. In chapter 8 the committee gives an overview of the effects on aquatic ecosystems and of those on terrestrial ecosystems in chapter 9. Finally, in chapter 10, the committee presents suggestions for preventive measures. The report is completed by a list of abbreviations, a list of references and the three annexes mentioned earlier, containing the full text of the request for advice, the make up of the committee and the report on the workshop.

Chapter 2

Exposure to UV radiation

In this chapter the committee gives an overview of various concepts and definitions regarding the quantification of UV exposure. It also indicates to what extent humans are exposed to sunlight and, more specifically, to ultraviolet radiation. There follows a discussion of the changes in the amount of UV radiation reaching the surface of the earth and the possible causes of these changes. Finally the committee makes predictions concerning the future UV climate, based on several possible scenarios.

2.1 Concepts and definitions

2.1.1 Subdivision of the UV radiation range

Ultraviolet radiation is a type of electromagnetic radiation. Electromagnetic radiation is characterised by the frequency (f), but the wavelength in vacuum (λ) can also be used instead of the frequency. There is a simple relation between the two quantities:

$$f = c / \lambda$$

where $c = 3 \cdot 10^8$ m·s^{-1}, the velocity of light in a vacuum. Figure 2.1 shows the different types of electromagnetic radiation, categorised by wavelength. As seen from the figure, ultraviolet radiation is defined as electromagnetic radiation with wavelengths between 100 and 400 nm. At the longer wavelengths the UV range is bordered by violet-blue light and at the shorter wavelengths by ionising radiation. The UV range is

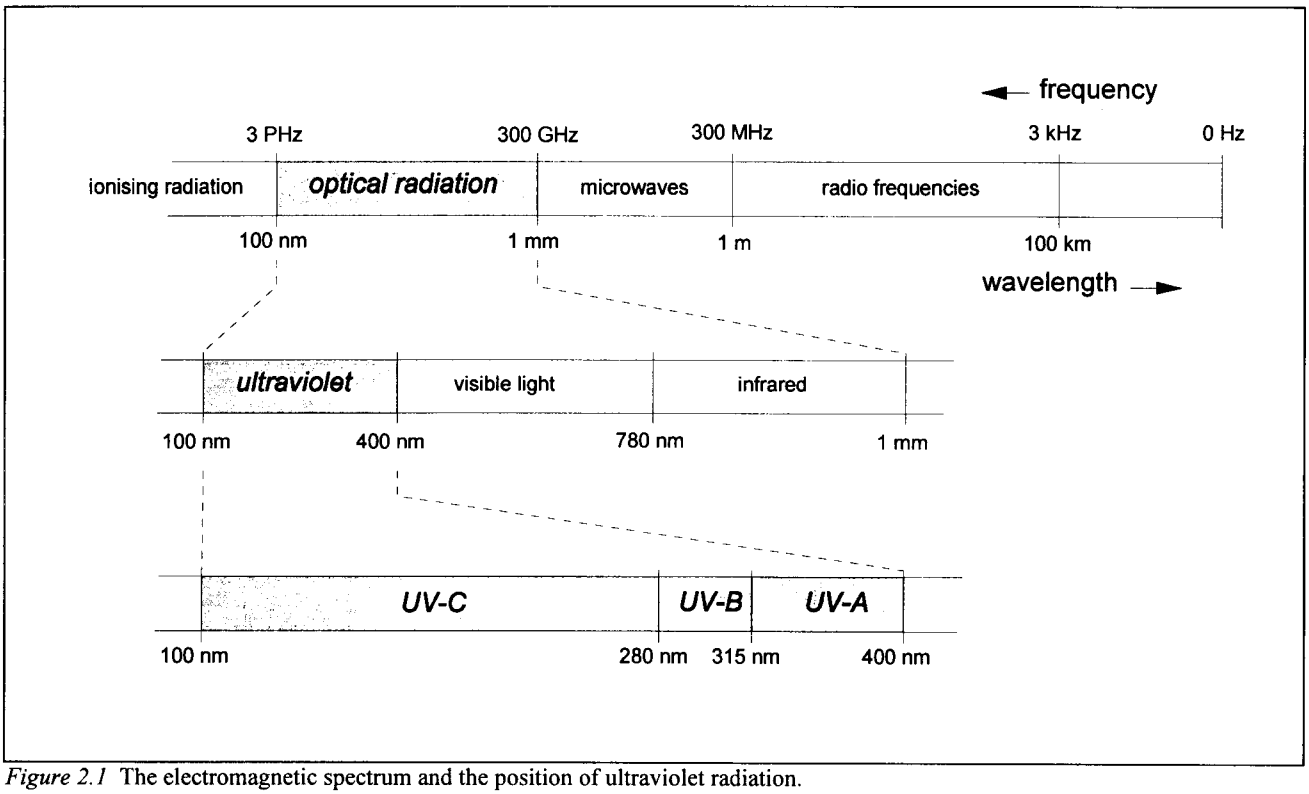

Figure 2.1 The electromagnetic spectrum and the position of ultraviolet radiation.

often subdivided into different wavelength ranges, since it has been shown for many UV effects that the amount of radiation necessary depends strongly on the UV wavelength. A division of the UV spectrum that is often used in photobiology is that into UV-A, UV-B and UV-C, with the limiting wavelengths (CIE70):

UV-C: 100-280 nm
UV-B: 280-315 nm
UV-A: 315-400 nm

In subsequent chapters the committee deals with a broad range of (possible) effects of UV radiation on humans and on the environment. It has been shown that, for many of these effects, this division into wavelength ranges gives a rough indication of the effectiveness of different UV wavelengths. The maximum effectiveness for a number of adverse effects of UV radiation, e.g. sunburn in humans and the development of skin cancer in animals, is in the UV-B or UV-C. The amount of UV-A required to obtain a certain effect is 1,000 to 10,000 times greater than that at the maximal effective wavelength in the UV-B or UV-C range. It is essential for the assessment of the effects of UV radiation to take into account the extent to which different wavelengths contribute to a given effect. Photobiology uses action spectra for the purpose. An action spectrum

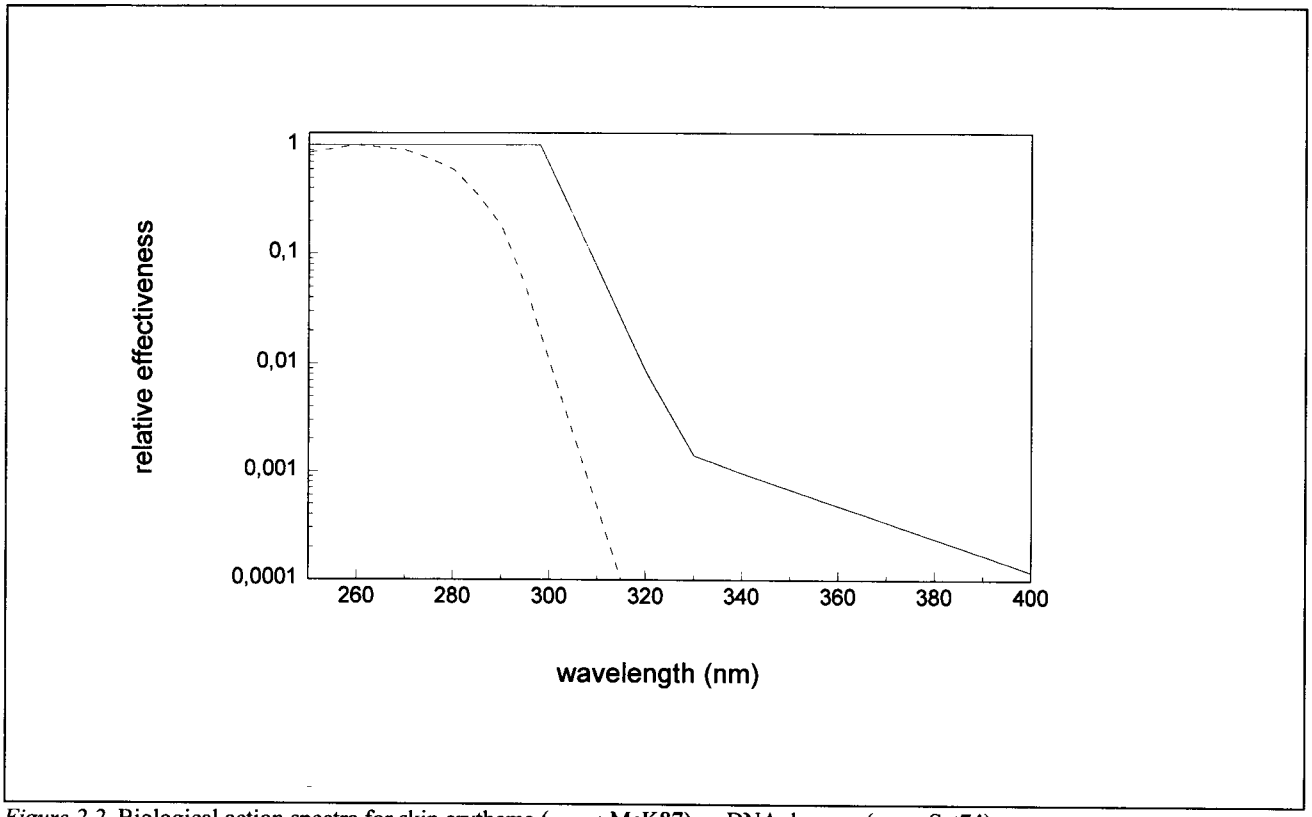

Figure 2.2 Biological action spectra for skin erythema (——; McK87) en DNA damage (- - - ; Set74).

can be considered as a series of wavelength-dependent weighting factors. At each wavelength the weighting factor is inversely proportional to the (monochromatic) dose necessary at that specific wavelength to cause the effect in question. An action spectrum is specific for the effect for which it has been determined, but many of the action spectra for biological effects look similar (figure 2.2). The committee will deal further with the use of action spectra in the determination of effective exposure in section 2.1.2.

In daily life UV exposure is almost always polychromatic, which means that exposure is to a broad wavelength band in the UV range. This is the case for both sunlight exposure and exposure to artificial UV or light sources. Estimation of the effects of polychromatic irradiation demands information about the spectral composition of UV that has to be combined with an appropriate action spectrum, and information about possible interactions of effects of different wavelengths. Physical quantities such as dose and irradiance have to be translated into effective units of exposure (see next section).

2.1.2 Quantities

Exposure is characterised by the spectral irradiance and the duration of irradiation. The spectral irradiance is the total amount of radiation energy reaching a surface unit per unit of time and per wavelength unit. The physical unit is $W \cdot m^{-2} \cdot nm^{-1}$. The spectral irradiation dose $H(\lambda)$ is obtained by integrating spectral irradiance over exposure time. The unit of spectral dose is $J \cdot m^{-2} \cdot nm^{-1}$. Several biological effects are related to the effective dose, which is calculated by weighting the spectral dose with the relevant weighting factor and integration over the entire wavelength range:

$$H_{eff} = \int H(\lambda) \cdot A(\lambda) \cdot d(\lambda)$$

where

H_{eff} = effective dose of UV radiation ($J \cdot m^{-2}$)
$H(\lambda)$ = spectral dose at wavelength λ ($J \cdot m^{-2} \cdot nm^{-1}$)
$A(\lambda)$ = weighting factor at wavelength λ (action spectrum; dimensionless).

The effective dose can be considered as the sum of the doses weighted per wavelength, where the weighting factors are taken from the action spectrum of the effect under consideration. The specificity of the action spectrum implies that the effective dose also is specific for the effect for which the action spectrum was determined. It is therefore necessary to indicate which action spectrum was used for the effective dose, and also to indicate the wavelength at which the relative effectiveness was set to unity.

The magnitude of the effective dose is important, but so is the period during which the dose was received. For short-term effects like sunburn (erythema) and snow-blindness the dose received within several hours to one day primarily determines the effect. For long-term effects such as the induction of skin cancer or a contribution of UV radiation to the development of cataract, yearly doses or perhaps even lifetime accumulated doses are important. With adaptation of the skin, and possibly immunological effects also, exposure lasting weeks or months is relevant. In order to evaluate the effects of alterations in exposure, the relation between the magnitude of the dose, the time and the extent of the effect must be known (the time-dose-effect relation) in addition to the action spectrum.

The effective dose, in combination with information on the time-dose-effect relation, allows the estimation of the effects of a polychromatic irradiation. Two conditions have to be met:

1 the dose-time-effect relation has to be known and be independent of the wavelength;

2 the interaction between the effects of different wavelengths has to comply with photo-additivity.

These two conditions mean that if a dose H_1 at wavelength λ_1 and a dose H_2 at wavelength λ_2 both result in the same effect,

- for all positive multiplication factors X the effect of a dose $X \cdot H_1$ at a wavelength λ_1 equals the effect of a dose $X \cdot H_2$ at wavelength λ_2;
- the combined irradiation with a fraction f of the dose H_1 and a fraction (1-f) of the dose H_2 will result in the same effect as each of the exposures separately (photo-addition).

The two requirements will certainly not always be met. Still, the concept of the effective irradiation dose functions very well in practice. It is always necessary to indicate which action spectrum is used and, therefore, for which effect the effective dose was determined. Information about the irradiation time is also essential.

2.1.3 *Exposure to effective UV: the standard MED (sMED)*

The minimal erythema dose (MED) is the radiation dose that results in a just perceptible skin erythema. It was noted in section 2.1.1 that the dose necessary for such erythema is highly dependent on the spectral composition of the light reaching the skin. This dependence can be accounted for by using the effective dose weighted according to the erythema action spectrum (see section 2.1.2). The erythema-effective dose corresponding to a MED varies from individual to individual and depends on, among other things skin type, skin area and the extent of adaptation of the skin resulting from preceding UV exposures. The MED can also be strongly influenced by the use of photosensitising substances. A representative reference value is often chosen for the MED and the erythema-effective exposure is expressed in MEDs. To avoid confusion with the individual MED, the committee will use the *standard Minimal Erythema Dose*, abbreviated sMED, as unit for the erythema-effective dose in this report. The committee uses an erythema-effective irradiation dose of 250 $J \cdot m^{-2}$ weighted according to the McKinlay-Diffey erythema action spectrum (McK87) as definition for the sMED. The 'Optical radiation' report of the Health Council of the Netherlands also uses this definition (GR93). This value for the sMED corresponds to the reference values for the minimal erythema dose proposed by international advisory boards.

Exposure to one sMED (within 8 hours) mostly leads to just perceptible erythema in caucasian individuals that have not been exposed to UV radiation for longer periods. In individuals with a skin that tans poorly and also does not adapt to UV very well (skin type 1, see section 6.8), e.g. red-haired, freckled people of Celtic origin, exposure to 0.3 to 0.5 sMED may be sufficient to lead to perceptible erythema. In persons with a

less sunburn-sensitive skin that has been adapted through regular exposures in preceding weeks and months, 3-5 sMED may be required for perceptible erythema.

2.2 Solar spectrum and sunlight exposure

2.2.1 *Solar spectrum*

The solar spectrum covers a broad spectral range that contains not only visible light, but also UV and infrared. The spectral composition of sunlight is highly influenced by the atmosphere. In the UV range this is especially the case for the UV-B and UV-C (see figure 2.3).

UV-C is completely absorbed in the atmosphere, while UV-B is strongly absorbed and scattered. Ozone (O_3) especially, most of which is located in the stratosphere at an altitude of 20-40 km above the surface of the earth, is an important absorber of UV-C and UV-B. Furthermore, UV radiation is scattered by atmospheric gases and aerosols. Since scattering increases with decreasing wavelength, UV-B radiation at the surface has an important diffuse component, even under clear sky conditions, in contrast to visible light. The ozone in the atmosphere is essential for a reduction of effective UV irradiance in the biosphere. UV transmission of the atmosphere depends on, among other things, the elevation of the sun, the total ozone thickness, the atmospheric distribution of ozone, the thickness, composition and distribution of aerosols and the cloud cover. The absorption in the atmosphere increases with increasing atmospheric pathlength of the radiation. Therefore, the spectral irradiation, and especially that in the UV-B range, depends strongly on the solar elevation. The solar irradiation in the UV-B range shows a large variation with season (see figure 2.4A) and day (figure 2.4B). Also, the total annual UV-B irradiation increases from higher to lower geographical latitudes; on the one hand as a result of the shorter atmospheric pathway, on the other because the average thickness of the ozone layer decreases at lower latitudes. The annual erythema-effective irradiation in Southern Europe (40° North, Madrid and New York) is estimated to be 50% higher than the effective irradiation in the Netherlands (52° North) (figure 2.4C).

2.2.2 *Exposure*

The total exposure to UV radiation from sunlight is related to the time spent outdoors, the orientation of the exposed surface and the possible reflection and shielding by factors from the environment and by clothing. Almost 1900 ± 300 sMED is available annually in the Netherlands, as measured at an unshielded horizontal surface (estimated on the basis of Sla87)*. However, only a small fraction of the maximally

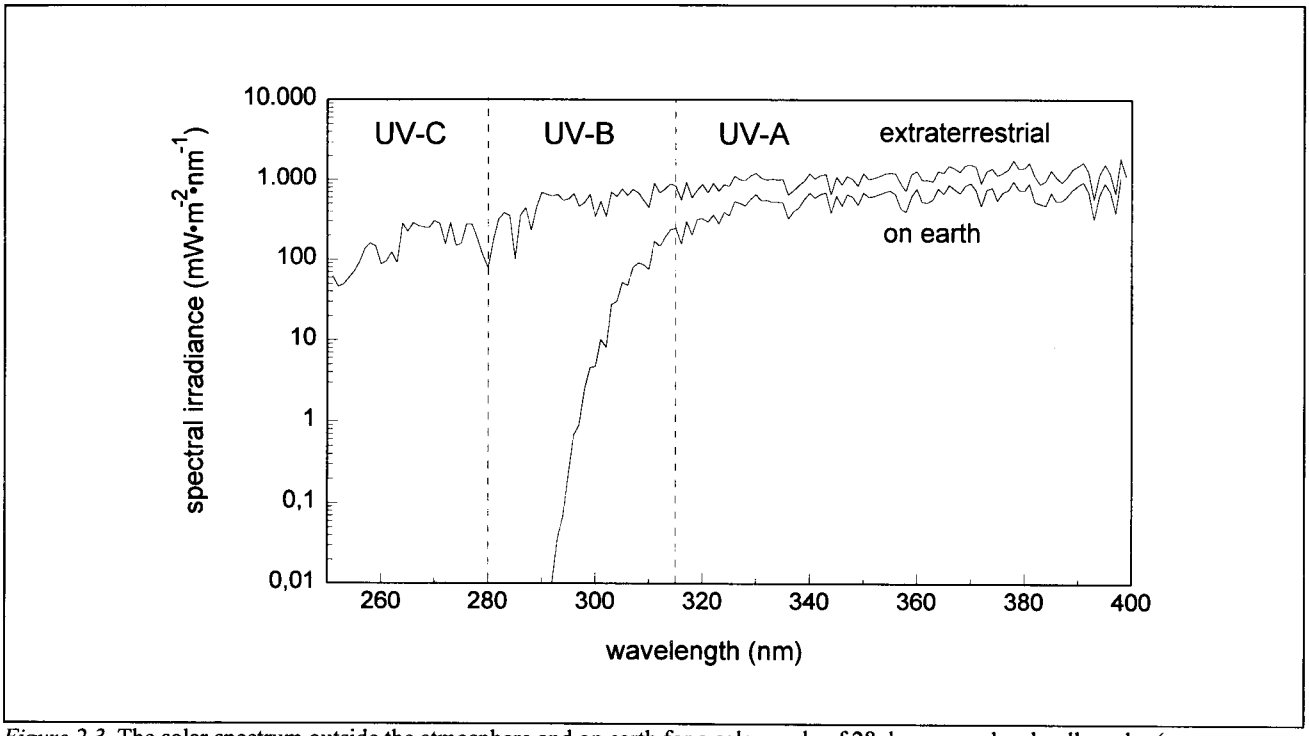

Figure 2.3 The solar spectrum outside the atmosphere and on earth for a solar angle of 28 degrees and a cloudless sky (measurement RIVM (Rei93); extraterrestrial spectrum: derived from satellite data).

available effective irradiation dose will reach the human skin or eye, or plants or animals.

Determination of the irradiances and the doses received is especially complicated for the eyes, because of the physiological shielding (size of the pupil, eyebrows, etc.), and for components in aquatic and terrestrial ecosystems, in which shielding and orientation complicate dosimetry.

Skin exposure

Although limited, some knowledge is available on UV exposure of human skin in the Netherlands. Based on data obtained with personal dosimeters it can be estimated that the sunlight exposure for indoor workers is approximately 2-3% of the effective dose available annually at a horizontal surface; for outdoor workers this is approximately

* Personal and environmental dosimetry was performed in Leiden, using polysulphone badges (Sch87). Based on these data the erythema-effective annual dose in the Netherlands was calculated, to be weighted with the Parrish action spectrum (Par82) (8 h following irradiation, normalised at 296 nm; Sla87). This results in an erythema-weighted annual dose on a horizontal surface of 45 ± 6 $J \cdot cm^{-2}$. The erythema-effective irradiation dose based on the McKinlay/Diffey action spectrum is approximately 1.07 times higher.

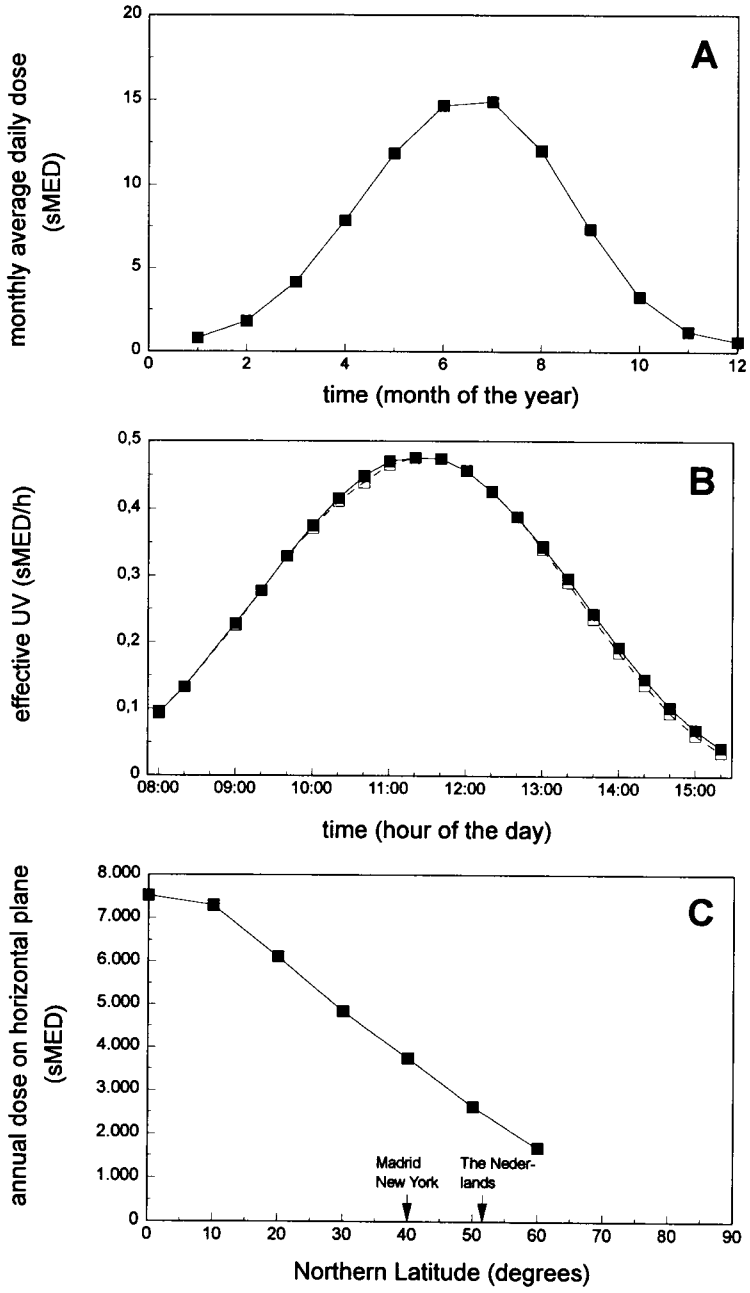

Figure 2.4 A: Seasonal variation of daily effective UV dose for clear days (model calculation RIVM (Lee88)). B: Daily course of effective UV irradiance under clear conditions (■: measurement 29 October 1993 (RIVM, Bilthoven, The Netherlands); □: model calculation RIVM; Slaper 1994, unpublished data). C: Latitude-dependence in the Northern Hemisphere of the effective UV irradiance (model calculation RIVM, based on clear conditions; reduction by cloud cover is estimated at 20-30% (Slaper 1994, unpublished data)).

7% (Sch87, Sla87). This pertains to the most commonly exposed areas of the hands and head. As a one-year total, indoor workers receive approximately 50 sMED (ranging from 40 - 60 sMED) and outdoor workers approximately 135 sMED. Exposure during holidays has not yet been included. A three-week summer holiday in the Netherlands may contribute approximately 20 - 40 sMED, and a similar one in Southern Europe 25 - 85 sMED. The mean sunlight exposure in the Netherlands is estimated at approximately 85 sMED annually, with a large behaviour-dependent spread.

The contribution of certain artificial UV sources, such as certain halogen desk lamps, tanning equipment or therapeutic UV equipment, to the annual UV dose can be considerable for people using these sources intensively. An estimate of the effective irradiance of halogen desk lamps gives approximately 0.2 sMED per hour at a distance of 30 cm from the source. Use of such lamps for 500 hours annually might therefore mean an effective exposure of approximately 100 sMED. In practice, the position of the lamp and of the exposed parts of the body greatly influence the dose received. Users of tanning equipment receive approximately 25 sMED annually, and the estimated group size is 7% of the total population (Bru87).

It is likely that at least 90% of the mean effective UV exposure in the Netherlands originates from the sun (Sla91).

The strong variation in the effective UV climate over the seasons, as shown in figure 2.4A, results in a large variation in exposure over the year. It is likely that this variation is amplified by exposure behaviour and by clothing: in the winter less time will be spent outdoors and more protective clothing will be worn. The highest effective irradiances occur around noon during the summer months: with the sun in the zenith maximally 2 - 3 sMED per hour can be received in the Netherlands, while maximum exposure to the winter sun does not result in more than 0.3 sMED per hour.

Exposure of the eye

No studies have been done in the Netherlands to investigate UV exposure of the eyes, and only limited knowledge about this subject is available elsewhere. It is clear, however, that the exposure of the eye is extremely complex. The location of the eyes in the orbits and the mostly downward look, in any case always away from the sun, result in the eyes being exposed primarily to dispersed and reflected UV radiation. Squinting and the pupillary reflex offer natural protection mechanisms to the eye against (damage by) high light intensities. These reflexes respond to visible light only, however, and therefore limit exposure to UV radiation only if there is a high visible light intensity at the same time. UV exposure of the eye is also influenced by the wearing of (sun)glasses and hats or caps.

Exposure of the eye is largely determined by reflected radiation and therefore the reflecting properties of the environment are important. The reflection of UV-B by grass and soil is relatively small, varying between 1 - 5%; water reflects 3 - 13%, light sand and concrete between 7 and 18%, but reflection on fresh snow can be as high as almost 90% (Sli86).

In the USA a retrospective study was performed regarding UV-B-exposure of the eye among Maryland Watermen. The median annual UV-B-dose on the eye in this group of outdoor workers was estimated at 2.2% of the total annual dose available on a horizontal surface (Tay88).

Exposure of aquatic ecosystems

The UV exposure of aquatic organisms decreases with increasing depth due to absorption and dispersion by substances dissolved in the water and by suspended material. Dosimetry is very complicated because there is almost always vertical mixing of the water column. Since algae, the organisms at the base of the aquatic food-chain, need visible light for their photosynthesis, the characteristics of UV exposure in the photosynthetic zone are especially important. Almost no data are available for the biologically relevant UV exposure of algae, or of any other organisms in the aquatic environment. It is clear, however, that there is considerable variability in light and UV exposure in surface waters. The penetration depth, defined here as the depth at which the intensity has decreased to approximately 1% of that at the surface, indicates the weakening of light by the water column. In surface waters in the Netherlands the penetration depth for visible light can vary from about 10 meters to about 10 centimeters. Pigments, e.g. chlorophyll, and suspended material cause absorption and dispersion of visible light and UV. Dissolved organic compounds, especially humic acids, specifically absorb UV radiation. With increasing depth high concentrations of humic acids result especially in a faster decrease of the UV component than of visible light. In clear sea water with low concentrations of humic acids, the penetration depth of UV is much greater and less than that of visible light by a factor of only about two. The UV exposure of plankton and other organisms, with the same irradiation, is clearly higher in the ocean than in surface waters in the Netherlands.

Exposure of terrestrial ecosystems

The UV exposure of terrestrial ecosystems depends strongly on shielding by plants in the direct environment. The fractions of UV available in an horizontal plane that are received by plants and animals, therefore, can vary considerably depending on the environment. Primary exposure will be to the upper parts of plants. Only scant data are

available for their UV exposure, but a number of plants will receive a considerably higher UV dose than humans as a result of their location. It is likely that changes in the radiation dose in a horizontal plane will lead to proportional changes in environmental UV exposure.

2.2.3 *Current trends in UV climate*

Knowledge about the present UV climate is limited. There have been no long range determinations of the spectral composition. Long-term trend measurements from 1974 until the present have been performed with Robertson-Berger meters (RB meters) (Sco88). The RB meter gives a spectrally weighted reading with a characteristic that has been slightly shifted to longer wavelengths relative to the erythema action spectrum. RB meters are, therefore, less useful for the analysis of the effects of ozone depletion, since it is the short-wave UV-B that is influenced by ozone. The trend measurements of Scotto (Sco88) give an indication of the decrease in UV irradiation at eight locations in the USA and Europe. This does not correspond to the increase that is expected due to the observed decrease of the average thickness of the ozone layer during that period (Sto91). It is possible that the positioning of the RB meters in the direct vicinity of airports in industrialised countries plays a role. In a cleaner environment, the Austrian Alps, Blumthaler, using RB meters, did find indications of an increase in UV-B irradiation (Blu90). Zheng and Basher came to the conclusion, based on a recent analysis of RB data for cloudless days in New Zealand (46° South), that the UV-B irradiation had increased by approximately 6% over the 1981-1990 period, while the depletion of the ozone layer was approximately 5% in the same period (Zhe93).

There are only limited data available from continuous recording of spectra. Spectral data have the advantage that evaluations based on different action spectra can be performed, at later stages also.

Spectral UV measurements in Antarctica have demonstrated that UV-B irradiance was clearly increased during passage of the 'ozone hole' (Sta92). Seckmeier and McKenzie (Sec92) observed that the effective irradiances in New Zealand (45° South) were greater than those in Southern Germany for comparable solar elevations. Most of the differences measured can be explained by differences in ozone layer thickness. They could not be explained by differences in tropospheric ozone and aerosol content. There are now indications of an increase in UV irradiation in the northern hemisphere, based on spectral measurements. For Toronto (Canada), from 1989 to 1992 the increase at a wavelength of 300 nm has been estimated at 35% annually during winter and 7% annually during summer (Ker93). In view of the low UV-B-intensity during winter (see figure 2.4A) the increase in summer is of more importance than that in winter for the total annual UV exposure.

There is no doubt that a reduction in stratospheric ozone, assuming an otherwise unaltered atmosphere, must lead to an increase in the amount of effective UV in the biosphere. It is not clear to what extent other changes in the atmosphere, specifically pollution of the troposphere with aerosols and ozone, will compensate for the reduced absorption by stratospheric ozone. If such compensation does occur, this cannot be expected to happen in relatively clean areas also. Moreover a coincidental compensation does not offer any guarantee for the future.

There are world-wide initiatives to improve UV monitoring. In the Netherlands, the National Institute of Public Health and Environmental Protection (RIVM) and the Royal Netherlands Meteorological Institute (KNMI) have started UV-monitoring. The RIVM concentrates its monitoring on the UV climate relevant to biological effects (Rei93). The measurements made in 1993 showed a clear relation between UV irradiation and the thickness of the ozone layer (KMI93). It will be 5 - 10 years before the series of measurements will provide insight into possible UV trends in the Netherlands. Based on the above-mentioned findings and on the recent data for ozone depletion in moderate latitudes of the Northern Hemisphere (see section 2.3.1) is seems likely that the UV-B load is increasing in the Netherlands also.

2.3 Model estimates of changes in UV climate

2.3.1 *Changes in ozone and the influence on effective UV*

Atmospheric ozone, most of which is located in the stratosphere, is the most important UV filter. Continuous production and destruction of ozone take place in the atmosphere. The thickness of the total ozone column, commonly expressed in Dobson Units (DU), is approximately 330 DU over the Netherlands, corresponding to a thickness of 0.33 cm at the surface at standard pressure and temperature. The thickness of the ozone layer fluctuates naturally according to season and, over the Netherlands and Belgium, is at its maximum (380 DU) in April and at its minimum (280 DU) in October (Mue92). The thickness of the ozone layer does not depend only on seasonal variations, but also on latitude (it is generally thinner over the equator than over the poles) and on meteorological conditions.

The possibility that human activities may lead to ozone layer depletion has been the object of discussion since the early seventies. Initially, this discussion focused on the role of high-altitude supersonic aircraft. Since the remarkable paper of 1974 by Rowland and Molina (Row74), however, attention was turned to the breakdown that might be the result of the large-scale emissions of chlorofluorocarbons (CFCs). These are very stable and have a long atmospheric persistence (tens to hundreds of years); they are broken down by UV radiation from the sun only after diffusion to the

stratosphere. In this process, chlorines are released that can break down stratospheric ozone. This may cause a disturbance in the balance between production and destruction of ozone.

The large-scale emission of CFCs during past decades led to an increase in the concentration of chlorine in the stratosphere. In 1985 it was discovered that the ozone layer over Antarctica was highly depleted in the early arctic spring: the 'ozone hole'. Re-evaluation of satellite data led to the conclusion that this 'hole' had developed at the end of the seventies. Further analysis showed that the depletion coincided with high chlorine concentrations in the area. The reason that this 'hole' appears specifically over Antarctica is related to the extremely stable atmosphere at the end of the arctic winter. There is little doubt that CFC emissions are the cause of the 'ozone hole'. The depletion in 1993 was again at a record low. The ozone levels decreased to 30% of the normal levels.

Considering the effects, it is relatively advantageous that the 'ozone hole' occurs in early spring and at high latitudes, because the low solar angle results in a relatively low UV irradiation. There is nevertheless reason for great concern. Moreover the depletion of the ozone layer is not confined to the Antarctic. Satellite observations of the past decade have shown world-wide ozone depletion (Sto91). Significant depletion has been observed in the Northern Hemisphere also. The general picture for the Northern Hemisphere is also that the strongest depletion is at high latitudes. No depletion is observed near the equator. The depletion that has been observed over the 1979 - 1990 period at the latitude of the Netherlands is approximately 7% in early spring and 2 - 3% during the summer period. The decrease in annual ozone levels over this period was approximately 5% (Her93a). This decrease is considerably greater than predicted. According to model estimates the increase in the annual UV load over this period should have been approximately 6 - 7% (Sla92). These estimates assume that the atmosphere remains otherwise unchanged.

Measurements at the Royal Meteorological Institute (KMI) in Uccle (Belgium), that has performed ozone measurements since the early seventies, do not fully confirm the strong depletion that has been deduced from the satellite observations for the eighties. Up to 1990 the decrease was limited. In recent years, however, record low values have been determined in winter and early spring, with monthly averages 15 - 20% lower than the long-term average (KMI93). It is very well possible, however, that the increased ozone depletion of recent years is related to the June 1991 eruption of the Pinatubo volcano in the Philippines. The massive amount of dust particles that reached the atmosphere could have contributed to the increased chemical breakdown of ozone by chlorines. It can be expected that the concentrations of dust will decrease in coming years. Recent data indicate that, in contrast to preceding years, no relative ozone depletion occurred in the early months of 1994. The chlorine concentrations will, however,

almost certainly continue to increase up to the beginning of the next century. Because of the slow breakdown, increased chlorine concentrations can be expected until late in the next century. It is likely that this will result in low ozone concentrations or even further depletion.

Since ozone absorbs primarily in the UV-B (and UV-C) range and hardly in UV-A, depletion of the ozone layer will result in a change in the solar spectrum at the surface of the earth. The magnitude of the increase of the effective UV-irradiation dose resulting from this spectral change depends on the action spectrum and therefore on the effect considered. The relation between the decrease in thickness of the total ozone column and the expected increase in effective UV is mostly indicated by the *optical amplification factor* that indicates the ratio between the percent increase in effective UV and the percentage depletion of the ozone layer. Table 2.1 gives optical amplification factors for several relevant effects (UNEP91). The value of the optical amplification factor equals the percent increase in effective UV dose for each percent decrease of the ozone column. The optical amplification factor depends on the solar angle and therefore also on the time of day*.

2.3.2 *Prognosis of the UV climate*

Recent scientific information on the depletion of the ozone layer has resulted in discussion at the international level regarding reduction of the emission of substances responsible for this depletion. Agreements have been made within UNEP to reduce the emissions of the most important CFCs. The Montreal Protocol, drafted in 1987, aims at a 50% reduction in the production of the most important CFCs by the year 2000. Given the alarming information about the Antarctic ozone hole and the surprisingly rapid depletion at moderate latitudes, the Montreal agreements have now been tightened up considerably twice. In London (1990), a complete production stop of the most important CFCs was agreed for the year 2000. In Copenhagen, in 1992, this production stop was shifted to 1996. At this time, agreements were also reached regarding the limitation of certain CFC replacement compounds. Figure 2.5 shows the production of the most important CFCs based on the agreements mentioned above.

Even in the case of a full world-wide compliance to the Copenhagen Amendments, rapid recovery of the ozone layer cannot be expected. This is the result of the long atmospheric persistence period of CFCs. Figure 2.6 shows the estimated increase in effective UV for the latitude of the Netherlands for different production scenarios

* It should be noted that, for high-percent ozone depletion, the increase in effective UV cannot simply be calculated by multiplication of the optical amplification factor by the percent depletion. In the evaluation of the annual totals, seasonal variations in the ozone column and in the ozone depletion also must be accounted for. These seasonal variations have been taken into consideration in the prognoses of the UV climate given in section 2.3.2 (Sla92).

Table 2.1 Optical amplification factors (VF_o) calculated for 30° North.

effect	VF_o		reference
	January	July	
mutagenicity and killing of fibroblasts	2.2	2.0	Zöl84, Pea84
DNA damage	1.9	1.9	Set74
generalised plant spectrum	2.0	1.6	Cal86
photosynthesis in leaf *Rumex patientia*	0.2	0.3	Run83
erythema	1.7	1.7	Par82
erythema	1.1	1.1	McK87
skin cancer (SCUP)[a]	1.4	1.3	Gru93b
cataract[a]	0.8	0.7	Pit77
immunosuppression[a]	1.0	0.8	DeF83
RB meter	0.8	0.7	Urb74

[a] determined in animal experiments

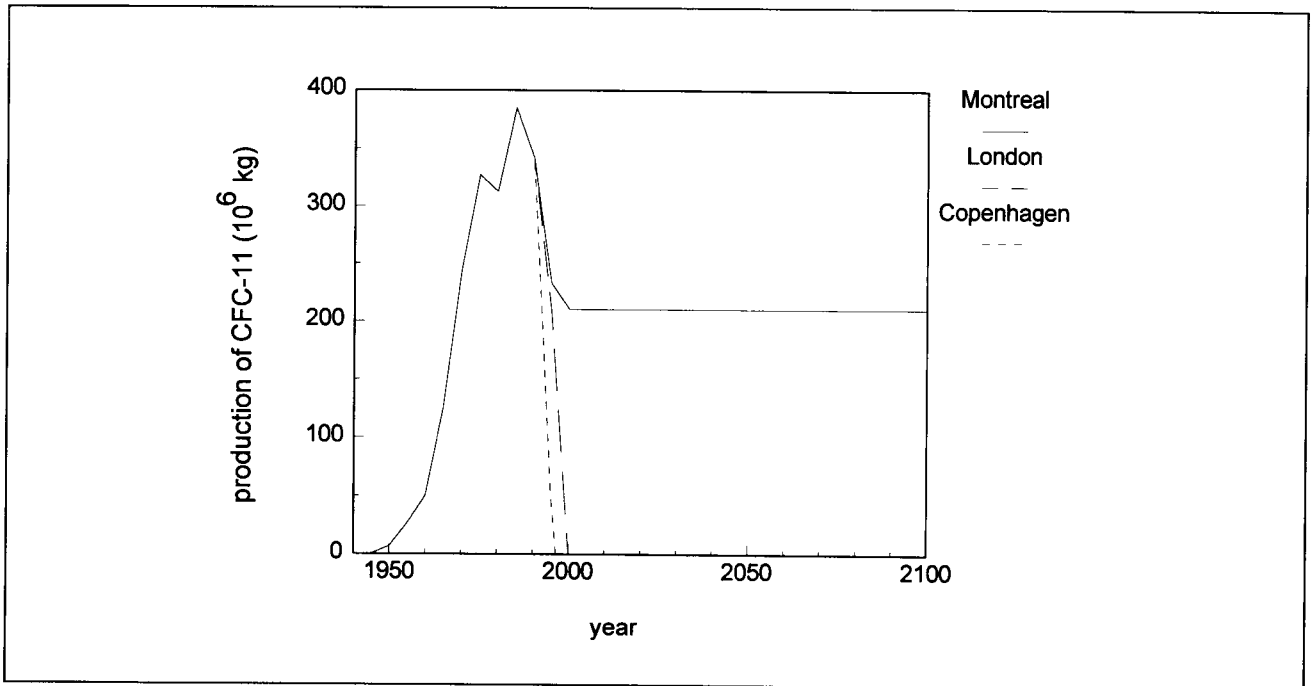

Figure 2.5 Scenarios for global CFC-11 production according to the agreements in the Protocol of Montreal and the London en Copenhagen Amendments (RIVM93, Sla92).

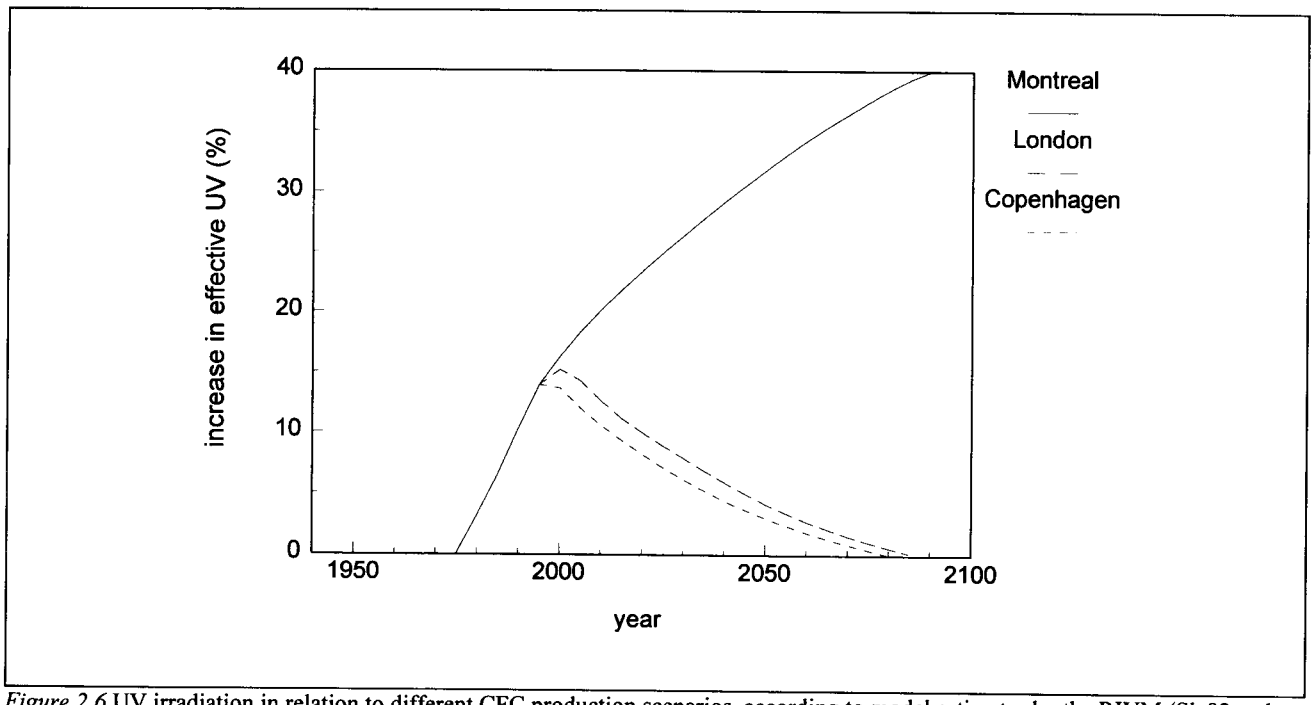

Figure 2.6 UV irradiation in relation to different CFC production scenarios, according to model estimates by the RIVM (Sla92; calculation based on the 'SteSla' action spectrum (Sla87)).

(estimates based on the RIVM UV chain model*). Full world-wide realisation of the UNEP protocols has been assumed and the ozone depletion at latitudes of the Netherlands calculated from satellite data was used. The observed seasonality of ozone depletion has also been accounted for. The results indicate the expected increase in effective UV. Over the 1979 - 1992 period the estimates obtained with this UV chain model agree with the estimates of Madronich and De Gruijl: an increase of approximately 9% of the carcinogenic UV irradiation dose (Mad93). The Copenhagen scenario results in an estimated increase in effective UV of approximately 15% at the end of the century. Slow recovery may then occur if a world wide production stop is indeed achieved.

2.4 Summary and conclusions

In order to evaluate the effects of exposure to UV radiation it is necessary to weight the contributions of the different wavelengths based on their effectiveness. The wavelength-dependent weighting factors (action spectra) are specific for the effect considered. For many effects the weighting factors of the shorter wavelengths (in the UV-B and UV-C range) are 1,000 to 10,000 times greater than those of the longer

* The calculations were performed with the UV chain model that evaluates the chain of CFC production, dispersion and chlorine load and the resulting ozone depletion, UV load and effects on the incidence of skin cancer (RIVM93, Sla92).

wavelengths of the UV-A range. The committee uses, unless otherwise stated, the standard Minimal Erythema Dose (sMED) as the unit for the effective weighted exposure. One MED is that dose that, in caucasian individuals with an unadapted skin, results in a just visible erythema. The sMED equals an irradiation dose of 250 $J \cdot m^{-2}$ at 297 nm and is weighted with the erythema action spectrum of McKinlay/Diffey (McK87).

The solar spectrum on earth contains, in the UV-range, primarily much long-wavelength radiation (UV-A). Nevertheless UV-B is the most important component of solar radiation, because of its much higher (biological) weighting factors for many UV-related effects. Solar UV-B is strongly absorbed by ozone in the atmosphere, primarily in the stratosphere.

The sun is the most important source of UV exposure for the population of the Netherlands and the only source for ecosystems and crops. In the Netherlands approximately 1900 ± 300 sMED is available annually, 60 - 70% of which in the May to August period. Exposure of the most-exposed skin parts, is estimated at 85 sMED annually for the Netherlands. There is a considerable spread in exposure related to variations in exposure behaviour. Outdoor workers receive annual doses two to three times higher than those to indoor workers.

No data are yet available on the development of UV irradiation in the Netherlands. In view of the recent observations that the ozone layer in the Northern Hemisphere is also affected, it must be assumed that UV irradiation has already increased in the Netherlands. It is likely that the increased chlorine load resulting from (global) CFC emissions plays an important role in ozone depletion.

Based on even the most favourable CFC production scenarios the chlorine load of the stratosphere will continue to increase, possibly resulting in even further depletion of the ozone layer. Based on model estimates an increase of approximately 15% in annual effective UV is expected in the Netherlands around the year 2000. After this development of the UV stress depends greatly on the achievement of the intended production stop. Full compliance with the agreements made in Copenhagen in 1992 might result in a slow recovery of the ozone layer after the turn of the century, but is likely that the UV irradiation level will still be elevated well into the next century.

Chapter 3

Interpretation of the results of epidemiological studies

The calculations and conclusions presented by the committee in chapters 6 and 7 that discuss the effects on skin and eyes are largely based on the results of epidemiological studies. The value of such studies depends, however, on several things, among them the way certain factors are accounted for. In the present chapter the committee identifies these factors and indicates how it has estimated the importance of the various studies.

3.1 Epidemiological methods

Epidemiology is a science that studies the occurrence of diseases in relation to the occurrence of factors that are expected to be somehow related to these diseases. The aim is to obtain indications as to possible causes of the diseases. Epidemiology is an observational, not an experimental science. It is not possible to give definite answers about a causal connection solely on the basis of results of epidemiological studies.

There are different approaches used in setting up and performing an epidemiological study. In the studies on the relation between exposure to sunlight or UV radiation and the occurrence of health effects described in this report, the following methods were used.

- Descriptive transversal studies of population groups. In such studies the prevalence of certain disorders in a population in relation to information about exposure to a certain factor is determined at a certain point in time.

- Prospective cohort studies. In such studies, exposure is the starting point. Two groups are set up: a group of individuals that are exposed to a certain factor and a matching non-exposed control group. The occurrence of diseases is observed for some time in both groups. A higher prevalence of a certain disease in the exposed group can be an indication of a causal connection with the exposure factor selected.
- Retrospective cohort studies. Data are gathered from a previous study that, initially, was not aimed at health effects of exposure to sunlight or UV radiation. As much as possible of the original population is traced, to study whether in the course of time a relation has emerged between certain disorders and exposure to sunlight or UV radiation.
- Case-control studies. In these studies the disease is the starting point. A group of patients is selected according to certain criteria and a group of controls is composed that matches as far as possible the group of patients regarding a number of relevant characteristics. Next, the factors to which patients and controls had been exposed in the past are identified. The finding that a certain exposure is more frequent among patients than among controls could indicate a possible causal connection.

Epidemiology depends strongly on statistical analysis of the data. The results can be expressed in units that give an estimation of the relative risk (RR). The RR is the ratio between the risk of occurrence of a certain disease in the exposed group and the same risk in the non-exposed group. If there is no influence of the factor in question the RR equals unity. Estimations of the RR are always associated with uncertainty that is expressed in the confidence limits determined for the estimate, using a certain confidence value (often 5% with unilateral testing). If the confidence limits do not include unity, the result is significant. This means that the statement 'the real relative risk differs from unity' has at most a 5% chance of being incorrect (in general: a chance equal to the confidence value selected). The relation then is statistically significant with 95% reliability.

3.2 Pitfalls in epidemiological studies

There are numerous problems in epidemiological studies that can decrease the validity or value (not to be confused with the statistical reliability) of the results. These problems can only be accounted for in part. The consequence is that the contribution of sunlight or UV radiation to the prevalence of certain health effects might be over- as well as underestimated. The committee took this into account and refers to these problems in a number of relevant cases.

In the sections following the committee presents an overview of the factors that may lead to incorrect interpretation of the results of epidemiological studies.

3.2.1 *Accuracy of exposure variables*

In studies on the effects of sunlight or UV radiation information on exposure is generally obtained from questionnaires. If exact recollection of exposure is at all possible, such a method allows at best a very crude estimate of the effective irradiation dose. An estimate of the exposure should pertain to the period that is biologically relevant for the effect under consideration. In the case of (skin) cancer or eye disorders such as cataract, cumulative exposure in relation to age and physical development is important regarding the development of the disease. It is very difficult, or virtually impossible, to determine individual exposure to UV radiation over longer periods (decades, preferably starting with childhood). The only type of study that allows actual measurements of exposure is the prospective cohort study.

In general, individual exposure can only be estimated semi-quantitatively, i.e., as crude levels. It is very likely that, in such quantifications, the so-called 'coincident errors' are considerable. In general, coincident errors in the estimation of exposure often lead to underestimation of the health effect in question. Coincident errors in the determination of interfering variables may also have an influence. In practice this may also lead to overestimation of the relation between exposure and effect.

In epidemiology, case-control studies are generally more highly regarded than descriptive transversal studies. Still, the latter may also provide valuable contributions to the study of the effects of UV radiation. Since UV radiation from sunlight can be adequately measured at certain geographical locations, UV-related disorders can be studied as a function of UV irradiation, e.g. at different latitudes. A prerequisite for doing this is that the behaviour and composition of the population at different locations be comparable. If these requirements are met, such studies have the advantage of allowing large numbers of individuals to be investigated and of offering good possibilities for the determination of a dose-effect relation.

3.2.2 *Accuracy of effect variables*

A variety of effect variables have been investigated in studies on the effect of sunlight or UV radiation. In the case of cancer the investigator most often depends on computerised records and on death certificates. These carry inherent inaccuracies, such as lack of information about disorders that were not the primary cause of death, or inadequate histological typing of tumours. Moreover, skin tumours are often removed without this being centrally recorded.

Coincident errors that are made in the determination of effect variables lead to a less accurate estimate of the relation between exposure and effect and to underestimation of the relative risk.

3.2.3 *Bias of the results by selection of study populations ('selection bias')*

When the selection of individuals for the groups to be compared is influenced by factors that are related to exposure to sunlight or UV radiation (in case-control studies) or with the disorder studied (in cohort studies), bias of the results may occur. This so-called selection bias may lead to both over- and underestimation of the risk attached to exposure.

3.2.4 *Biased observation or reporting ('information bias')*

The information used for the assessment of exposure or effect variables can be incorrect for some of the study groups as a result of bias of the study participants or of the researchers. This may lead to bias of the results.

In case-control studies, knowledge about the relation between disease and exposure to sunlight or UV radiation might knowingly or unknowingly have affected the information concerning exposure that was given by patients or their relatives. Skin cancer patients may recall their exposure better than do controls. There is also the danger that investigators question patients more assiduously than controls about their exposure.

In cohort studies the detection of disorders by the investigator may, knowingly or unknowingly, be influenced by his knowledge about the exposure of the individual under study.

3.2.5 *Confounding variables (leading to 'confounding bias')*

In addition to the factor under investigation there are often many other factors that might influence the development of the disease. If these factors are somehow connected with exposure to sunlight or UV radiation, the relation between exposure and the health effect in question may be confounded. These factors are then called 'confounders'. Confounding can be limited by gathering data on the influence of known confounders and applying a correction for this influence.

In the studies on the relation between sunlight exposure and the development of skin cancer, skin type (light or dark, see chapter 6) is an important confounder. Individuals with a naturally dark skin can stay longer in sunlight without developing sunburn. They are also considerably less susceptible to skin cancer.

3.2.6 *Bias by selective publication of results ('selection bias')*

Selective publication of results that indicate a negative effect of exposure may lead to an overestimate of the true risk in the assessment of published results. Editorial boards of scientific journals may tend to accept those manuscripts that present results indicating a relation between exposure and effect more easily than those that present results indicating the absence of this relation. There are no indications of publication bias, however, for the studies on the effects of exposure to sunlight or UV radiation.

The researchers themselves may have focused specially on indications of a negative effect when they tested the collected data and did not consider publication until they found such indications.

3.3 Criteria for judging the results of epidemiological studies

In judging the results of epidemiological studies the committee not only considered the magnitude of the relative risk, but also the extent to which a result might have been due to chance. It also examined to what extent the sources of bias mentioned above might have played a role.

Epidemiological studies are essential to determine whether a dietary or environmental factor really is associated with a certain disorder. Criteria have been adopted in epidemiology to judge the possibility of a causal relation between exposure to a factor and the prevalence of disorders (Hil71, Kle82). To conclude this chapter the committee briefly describes these criteria.

Biological plausibility

A causal relation between exposure to a factor and the prevalence of a certain disorder is more likely if a possible biological mechanism is known. With regard to the development of skin tumours, the damage inflicted upon DNA by UV radiation and the subsequent processes leading to the formation of a tumour have become clearer (see chapter 6).

When supporting biological views are lacking, the results of epidemiological studies are considered as 'hypothesis-generating'. Further research then will have to show whether the hypothesis can be accepted or should be rejected.

Consistency

The likelihood of a causal relation increases when a relation between exposure and a health effect is found in different study populations under different circumstances.

Strength of relation

The strength of the relation, expressed as the relative risk, is a measure of the importance of the factor under investigation. The higher the (statistically significant) relative risk observed, the less likely it is that the relation completely results from chance.

Temporality

Exposure to sunlight or UV radiation can only be considered a causal factor when the time between exposure and the occurrence of health effects corresponds to a reasonable extent with the latency time. Especially in transversal and case-control studies, it is often difficult to establish the exact temporal order of exposure and effect.

Dose-response relation

If a connection between the extent of exposure and the intensity of the response is observed, this generally supports the conclusion that there really is an effect of exposure. One has to account, however, for the possibility that, as exposure increases, the influence of sources of bias and of confounding factors may also increase.

Knowledge of a possible biological mechanism, however, is essential for the assumption of a causal relationship.

Chapter 4

Photochemical reactions in tissues

4.1 Absorption of light and UV radiation

The absorption of light and UV radiation by certain molecules may lead to chemical or physical changes in a cell. The range of wavelengths in which photon absorption takes place depends on the structure of the specific molecule. When the absorption of photons has put the molecule in an excited state, a reaction takes place if enough energy is absorbed to overcome the activation energy for that reaction.

Molecules that are excited photochemically can act both negatively and positively on cells and organisms. For instance, photosynthesis, the basis of life, starts with the absorption of visible light by chlorophyll in plant cells. In human skin cells, UV radiation induces the formation of (pre)vitamin D_3. On the other hand UV radiation may cause damage to the DNA in the same cells, that ultimately may lead to the formation of a tumour. Cells, organisms, and ecosystems both depend directly and indirectly on useful photoproducts for their existence, and have developed sometimes complicated mechanisms for the repair of photochemical damage and the breakdown of noxious photoproducts.

4.2 UV-absorbing biomolecules

Important UV-absorbing biomolecules include nucleic acids (DNA and RNA), proteins, urocanic acid, pigments (e.g. melanin) and, in plants, chlorophyll, anthocyanide and flavonoids.

From a biological point of view, DNA, the molecule containing the genetic information, is a vulnerable part of the cell with respect to exposure to UV radiation. It is essential for the functioning of the cell that the DNA should remain intact. In irradiation of mammalian cells with wavelengths shorter than 310 nm, the amount of absorption by the DNA correlates with cell death, the induction of mutations (permanent alterations of the DNA) and cell transformation (changes in the cell leading to uncontrolled growth). UV radiation is strongly absorbed by DNA and RNA as a result of the aromatic ring structure of the bases, the building blocks of these molecules (see section 4.3). Maximum absorption is centered around a wavelength of 260 nm. Although absorption decreases strongly at wavelengths over 280 nm, it is still considerable at 300 nm. Absorption by proteins is mainly in the UV-B range and results from the aromatic amino acids (tryptophan, tyrosine and phenylalanine). Their extinction coefficient, however, is an order of magnitude less than that of the nucleic acids. Since proteins are present in greater quantities, more UV radiation is absorbed in the absolute sense by proteins than by nucleic acids. Organic molecules with unsaturated bonds like flavins, porphyrins, quinones and carotenoids also absorb in the UV range. Therefore they, too, can be involved in the biological effects of UV radiation.

Following the absorption of photons, the energy can be transferred to other molecules. This may disrupt chemical processes and damage biological systems such as membranes. Many pigments exhibit this mechanism of energy transfer. Such so-called 'photosensitisers', e.g. the flavin-containing compounds, often occur naturally.

4.3 DNA damage

Exposure of DNA to UV radiation results in various types of damage. DNA is built from smaller units, nucleotides, each consisting of a deoxyribose(sugar)-group and a base, that are coupled by phosphate groups. There are two types of pyrimidine bases (thymine and cytosine) and two types of purine bases (adenine and guanine). Subsequent to UV absorption, alterations occur primarily in pyrimidine bases. Photoproducts are formed that consist of an abnormal binding of two pyrimidine bases located side by side in the same DNA strand. The most frequently occurring photoproducts are cyclobutane pyrimidine dimers (figure 4.1). To a lesser extent pyrimidine(6-4)pyrimidon photoproducts and 'dewar' isomer photoproducts are also formed. A number of other photoproducts are known, but these occur only very rarely. An action spectrum is available for the formation of cyclobutane pyrimidine dimers and pyrimidine(6-4)pyrimidon photoproducts in mammalian cells (Cha86, Ros87). These are primarily formed by UV radiation with wavelengths shorter than 310 nm. At longer wavelengths DNA single strand breaks are relatively prominent. These are also induced by radicals, highly reactive molecules such as singlet oxygen and the OH

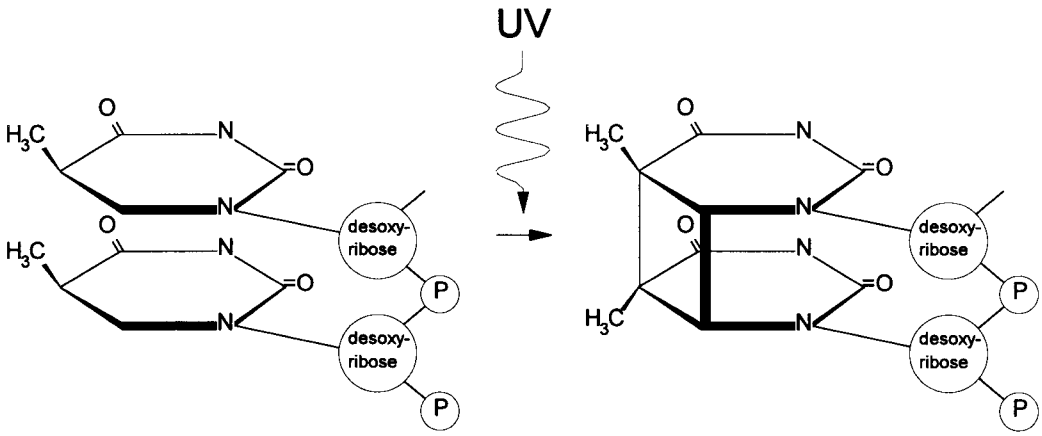

Figure 4.1 Schematic representation of the formation of a thymine dimer. UV radiation induces the formation of a cyclobutane ring between two thymines located side by side.

radical (Roz85). Free radicals can also induce links between proteins themselves and between DNA and proteins.

4.4 Protective mechanisms

Cells have various mechanisms available for protection against excessive damage. These can be divided into mechanisms that prevent damage and mechanisms that repair damage.

One way to prevent damage is to eliminate reactive oxygen radicals. Several compounds act as radical scavengers. One of the most important of these is glutathione, which is present in high concentrations in the nuclei of mammalian cells. The thiol group of this molecule scavenges radicals and singlet oxygen. Glutathione depletion in cells results in decreased cell survival following UV-B irradiation (Tyr86, Tyr88). Other naturally occurring radical scavengers are ubiquinol, vitamin C (ascorbic acid) and vitamin E (α-tocopherol).

In the skin, protection is also offered by pigment. The presence and formation of melanin is one of the factors that determine the sensitivity of the skin to sunlight. There are two types of melanin: eumelanin (darker) and pheomelanin (lighter) (Pav93). It is especially the amount of eumelanin that determines the degree of protection. This compound can absorb UV radiation directly as well as scavenge radicals that

have been released from other molecules by UV radiation. These processes do not result in cellular damage. Interaction of UV radiation with pheomelanin, prominent in red-haired individuals, on the contrary can possibly produce oxygen radicals and therefore damage molecules which are important for cellular functioning (Pan92) (see also section 6.8).

Flavonoids are very common in plants. The synthesis of these protective pigments can be up-regulated on exposure to UV radiation, depending on the properties of species and cultivars (DSu93) (see also section 9.2). Their biological activity is generally described as anti-oxidation.

Membrane damage is mostly abolished because membranes are in a semi-fluid state and are therefore able to quickly repair damaged parts by synthesising the membrane-constituting proteins and lipids. In the case of severe membrane damage, degeneration of the cell and removal from the tissue occurs. In the lens of the eye, where removal is impossible, membrane defects are encapsulated.

If damage has been inflicted upon the DNA, in spite of the above-mentioned protective mechanisms, the DNA needs to be repaired as rapidly as possible in order to ensure cellular functioning and growth regulation. Various enzymatic mechanisms are available for the repair of UV damage. The most important ones are nucleotide-excision repair and photo-repair. A number of enzymes or enzyme complexes are involved in nucleotide-excision repair. They all have a specific role in the subsequent detection and removal of the damaged DNA sequence, the synthesis of new DNA and the repair of the DNA strand.

Photo-repair involves the repair of the DNA damage by a single enzyme complex (photolyase), that needs to be activated by short-wave visible light. This repair mechanism has been demonstrated in various cell types. The only mammalian cells that have this mechanism are opossum cells.

In chapter 6 the committee indicates to what extent, and how, specific DNA repair takes place.

4.5 Phototoxicity

Certain hereditary metabolic disorders are characterised by cellular accumulation of compounds that increase sensitivity to UV radiation. An example is the accumulation of porphyrins in genetically determined defects in the synthesis of haem-containing compounds. It is also known that certain drugs and chemicals can be toxic to cells and organisms or cause (photo)allergic reactions after absorption of visible light or UV radiation. Para-aminobenzoic acid, a compound that is often used in sunscreens because of its UV-B absorptive properties, may cause allergic reactions. Other substances, for instance some used in cosmetics, can bind to proteins and nucleic acids as a result of

UV-B absorption. This may result in toxicity and in a strong increase in the sensitivity to UV radiation.

Chapter 5

Effects on the immune system

5.1 Introduction

The immune system protects the organism against 'attacks' from outside, for instance infectious agents (bacteria, viruses, parasites, molds) and from inside (formation of tumours). Especially at the interfaces of the organism and the outside world there is continuous exposure to many potentially pathogenic agents. Physical and mechanical barriers are the first line of defense against these pathogens. In addition to this, the immune system is active. Effects on the immune system may have implications for resistance to tumours, but may also have adverse effects on the possibly even more important function of the immune system, resistance to infections.

In this chapter the committee explains briefly the structure and functioning of the immune system, dealing specifically with some aspects of the immune system in the skin, it indicates the consequences of suppression of the immune system and finally summarises the effects on immune parameters in experimental animals and humans and the consequences of these effects for the resistance against infections and tumours. Evaluation of this information will include a discussion of the risk increasing exposure to UV radiation poses for the general population and for groups specifically at risk. The committee will present recommendations for dealing with these risks and what type of research is needed to better map them.

5.2 The immune system

5.2.1 *Specific and non-specific resistance*

The immune system can be divided into the non-specific and the specific immune system.

The non-specific immune system is based on several mechanisms: phagocytosis and intracellular lysis (ingestion and digestion of the pathogen in the cell) and intracellular cytotoxic activity (killing of infected cells by special cell types). These mechanisms are directed against the invading pathogen in a non-specific fashion. Together with the physical and chemical barriers, they fulfil an important function in the first line of defense.

Specific resistance develops some time after the first contact with a pathogen and is able to stimulate and complete the non-specific resistance. This type of resistance is directed against specific non-self compounds (mostly proteins). These compounds are called antigens. Specific resistance is important for accelerated defense in the case of re-infections (memory function).

5.2.2 *Tissues, cells and mediators of the immune system*

The immune system is not located in specific organs, but its components can be found throughout the body (figure 5.1). The structure of the immune system is very complex and extremely balanced. For a better understanding of the effects that will be discussed later, the committee now presents a short description of the most important features.

Cells

All cells of the immune system, the leukocytes, differentiate from pluripotent cells, either through the lymphoid pathway (lymphocytes) or through the myeloid pathway (phagocytes and other cells) (figure 5.2). In the primary lymphoid organs, bone marrow and thymus, lymphocytes replicate independently of the presence of antigen.

Humoral and cellular immunity

Humoral immunity is not a direct result of cellular action, but depends on antibodies in the blood. These antibodies are certain proteins that are produced by plasma cells (mature B-cells). T-lymphocytes regulate humoral immunity for certain (T-cell-dependent) antigens.

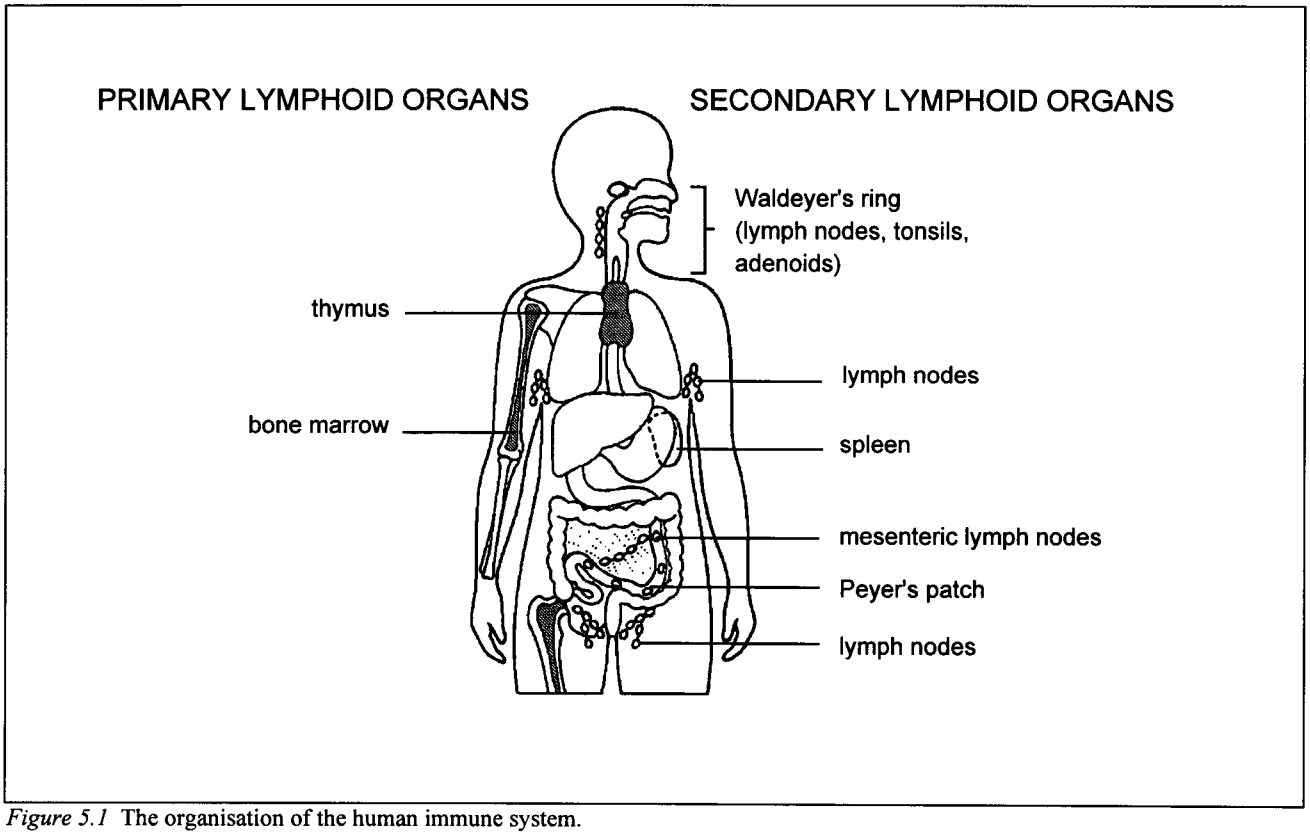

Figure 5.1 The organisation of the human immune system.

Cellular immune responses are specific reactions that are not caused by humoral factors but result directly from the action of T-cells.

Different T-cells can be distinguished. T-helper cells can help regulate the production of antigen by B-cells. They can also stimulate non-specific effector cells. They exert their activity by producing interleukins. These are low-molecular weight compounds that can stimulate other cells to certain activities. Finally there are the cytotoxic T-cells that are capable of killing tumour cells and virus-infected cells. Cytotoxic T-cells can also suppress the activity of other T-cells, and are therefore also called suppressor T-cells.

The immune system calls into action in the immune response several cell types that are not capable of recognising antigens. Among these non-specific cells (figure 5.2) are macrophages, natural killer (NK) cells, neutrophilic, eosinophilic and basophilic granulocytes, mast cells and probably even endothelial and epithelial cells.

Macrophages and neutrophilic granulocytes can phagocytose non-self material and are therefore suitable for functioning in the first-line defense against bacteria. Tumour cells and virus-infected cells are more difficult to phagocytose. The first line of defense against these latter cells is formed by cells with NK activity. Lymphoid cells

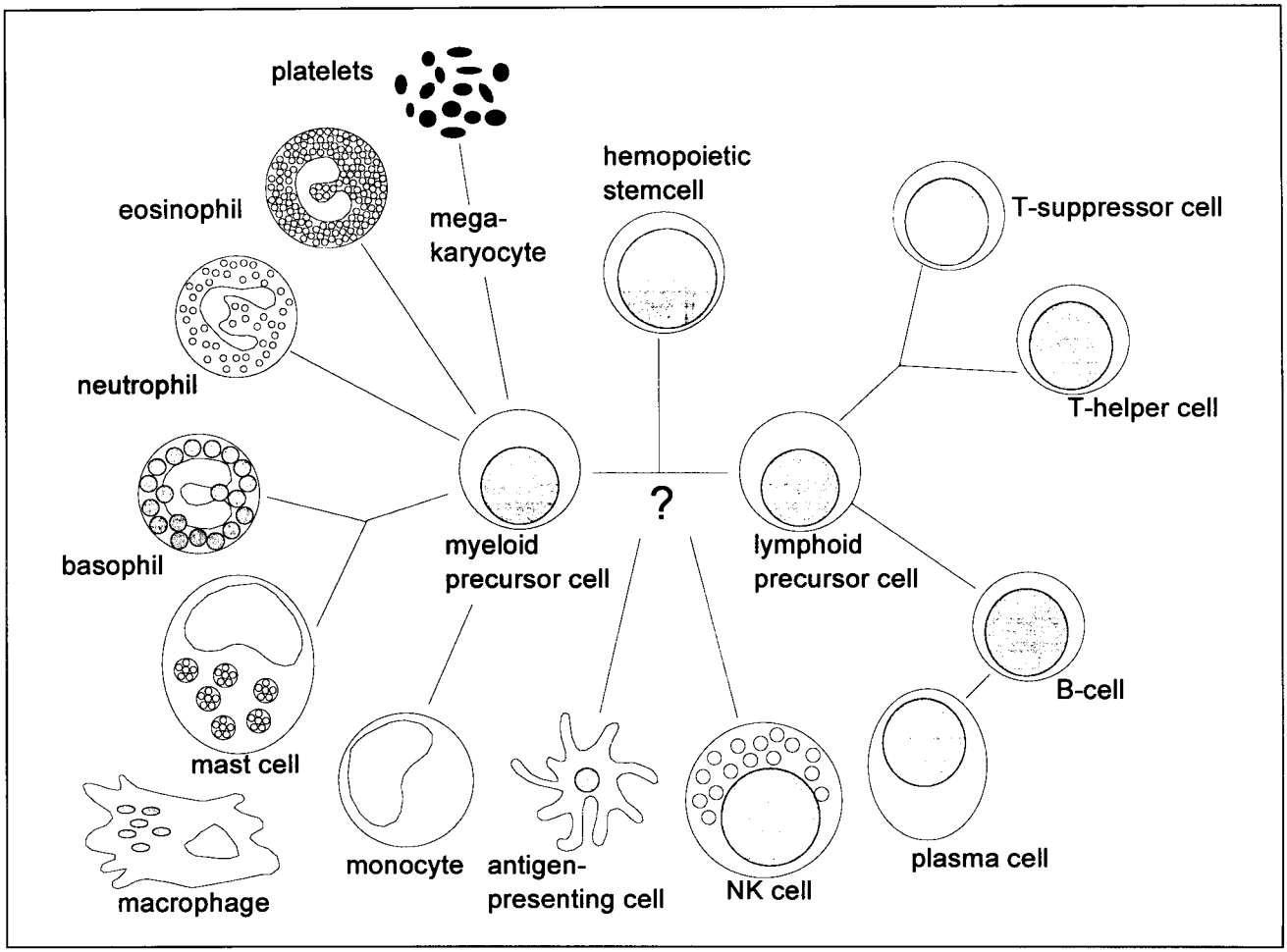

Figure 5.2 Cells of the immune system.

with this activity, as well as cytotoxic T-cells, can kill these target cells by means of an extracellular process. In contrast to T-cells, they do so without being sensitised and therefore aspecifically.

5.2.3 *Effector mechanisms in the immune system*

Macrophages are often the first defense against intruding bacteria. Macrophages can present bacterial antigens to lymphocytes after phagocytosis of the bacteria. These lymphocytes then can direct macrophages to the location of the inflammation by means of signal compounds and stimulate macrophages to increase their activity. This process can terminate the infection.

Virus-infected cells and tumour cells are initially attacked by NK-cells, assisted by macrophages. Both cell types secrete compounds that are toxic to the target cells

which are therefore lysed by an extracellular process. During this process antigens are released, that are presented to lymphocytes by antigen-presenting cells. This mechanism activates cytotoxic T-cells, that also lyse target cells.

In general, effector T-cells are activated by penetration of antigen into the organism and presentation of the antigen to the lymphocytes by antigen-presenting cells. This interaction results in 'memory' for this antigen. In this way the immune system can react quickly and violently on renewed contact with the same antigen in order to remove the source of the antigen as rapidly and adequately as possible.

Auto-immunity and hypersensitivity

Effector mechanisms aimed at damaging the pathogen can often also result in damage to autologous tissues. When this type of damage dominates over damage to the pathogenic or non-self agent that the immune system is attempting to eliminate, auto-immunity, or hypersensitivity comes into play.

In auto-immunity, the immune responses are specifically directed against components of one's own body. There are different forms of auto-immunity, depending on the product or agent reacted upon and on the type of immune response.

Hypersensitivity also is of different types. The most important are direct-type and delayed-type hypersensitivity. In direct-type hypersensitivity an important role is played by plasma cell-produced antibodies that are specific for the allergen against which hypersensitivity exists. These proteins, IgE-type immunoglobins, bind to mast cells and basophilic granulocytes. Interaction of this complex with the antigen results in rapid (within minutes or hours) secretion of mediators by the mast cells. These compounds can result in tissue damage which, in the skin, can manifest itself as urticaria. With delayed-type hypersensitivity an adverse inflammatory reaction occurs only long after the contact with the allergen. In the skin, this can manifest itself as eczema. Contact hypersensitivity is a delayed-type hypersensitivity. In delayed-type hypersensitivity reactions, T-lymphocytes act as mediators. Although delayed-type hypersensitivity is an adverse reaction, the ability to respond in this way is a measure of the function of the immune system. The effects of UV-B radiation on the immune system are determined experimentally by means of delayed-type hypersensitivity reactions.

A properly functioning immune system is also very important for resistance against opportunistic infections (caused by pathogens already present in the host, but only resulting in disease when resistance against them decreases) and against neoplastic lesions (cancer).

5.2.4 *The immune system in the skin*

Similarly to other locations in the body that are in contact with the external environment, components of the immune system are located in the skin. The Langerhans cells in the epidermis are very effective in the presentation of antigen to T-lymphocytes, the first step to a primary immune response. Keratinocytes in the epidermis can produce interleukins that can influence the differentiation of T-cells.

These cell types constitute more or less independent immune regulatory pathways in the skin. Changes in the immune system in the skin, however, may affect the immune system in other parts of the body, because there is continuous recirculation of lymphoid cells from the skin to the draining and other lymph nodes. Recirculating lymphocytes are the basis of 'immune surveillance' and as such are very important in the resistance against pathogenic agents and tumour cells.

5.2.5 *Immune suppression*

The result of a reduction in the resistance against infections and neoplastic lesions is probably best illustrated by the dramatically increased incidence of lymphomas and leukaemias, Kaposi sarcomas and opportunistic infections as seen in AIDS patients (acquired immunodeficiency syndrome). The reduced resistance in these patients results from infection with the HIV (human immunodeficiency virus) that is disastrous for the immune system. In these patients, the activity of the immune system and the number of Langerhans cells in the skin are drastically reduced. Wart-like lesions with a viral background are one of the first skin symptoms in these patients. Skin tumours develop later.

Further indications have been found during the last decade that human and animal exposure to external factors influencing the immune system results in a higher frequency of infections and neoplastic lesions. This is especially apparent when certain drugs are used. Organ-transplant patients that are treated for long periods with immuno-suppressive drugs to prevent rejection of the graft show a markedly increased incidence of skin tumours (see section 6.8.1).

Although it is likely, based on animal experiments, that even modest immune suppression will result in an increase in the incidence of infectious diseases and tumours on a per population basis, this has not been unequivocally demonstrated.

5.3 Immune suppression by ultraviolet light

UV irradiation of the skin may result, among other lesions, in alterations in proteins of the cell nuclei or in membranes. These altered proteins may be experienced by the organism as non-self. The immune system may react with an defensive reaction to these so-called photo-antigens. Since UV radiation can also induce antigen-specific immunosuppression, there is no excessive or prolonged inflammatory reaction after UV irradiation.

The immune suppression induced by UV radiation comprises mainly suppression of the cellular immune responses by, e.g., influencing the presentation of antigen by Langerhans cells. Such immune suppression will of course first become manifest in the irradiated skin, but systemic immune suppression will also occur subsequently.

5.3.1 *Influence of UV-B on the distribution of lymphocytes*

Data from animal experiments indicate that the immunosuppressive effect of UV radiation can partly be the result of changes in the distribution of lymphocytes. An altered pattern of recirculation and migration to lymph nodes has been observed in mice exposed to UV-B radiation for six consecutive days and subsequently injected with marked lymphocytes. Compared with non-exposed animals, more lymphocytes appeared in peripheral lymph nodes of these mice. This was observed up to two months after exposure (Spa83). The number of leukocytes had decreased.

Recently it was shown that exposure to UV-B can suppress the expression of cellular adhesion molecules such as ICAM-1, which is primarily present in the cell membrane of keratinocytes and Langerhans cells and is involved in the migration and recirculation of lymphocytes in the skin (Nor89, Tan91).

5.3.2 *Effects of UV-B on Langerhans cells*

It has been demonstrated that, in experimental animals, exposure to UV-B can also influence antigen presentation by Langerhans cells (Sti81). Under normal circumstances Langerhans cells produce a signal that leads to activation of receptors on T-helper-1 (Th1) cells. This then results in an immune response. Through blockade of the Th1 activation signal, exposure to UV-B can result in the absence of a reaction to antigen. Also, the production of interleukins by Th1 cells (e.g. interferon-γ, IFN-γ) can be suppressed through the secretion of interleukin-10. Th1 cells initiate delayed-type hypersensitivity responses through secretion of IFN-γ, one of the most important mediators associated with effector functions of the immune system.

In addition to these local effects there can also occur systemic effects. The altered Langerhans cells migrate to draining lymph nodes, where they influence the recirculation of T-lymphocytes.

Langerhans cells are not always involved in suppression of the immune system. Both a decrease in the number of Langerhans cells (Oba85) and suppression of contact hypersensitivity (Toe80) have been observed after exposure of the skin to UV-B, but the wavelength-dependence of these reactions differs (Noo84).

A reduction in the number of Langerhans cells following UV-B irradiation has also been observed in humans. After the disappearance of the Langerhans cells, suppressor T-cells appear in the epidermis. These cells are also capable of presenting antigen to other lymphocytes, but they can also induce immunosuppression (Baa90).

5.3.3 *Effects of UV-B on keratinocytes*

Animal experiments showed that keratinocytes play an important role in the UV-B-induced effects on the immune system. Production and secretion of the different interleukins and other mediators by keratinocytes is stimulated by UV-B irradiation. Of prime importance is the secretion of tumour necrosis-factor-α (TNF-α). This compound is partly responsible for the suppression of contact hypersensitivity responses (Ver90).

The mechanism of the effects of UV-B on keratinocytes is not yet clear. Damage to the DNA, but also effects on the cellular membrane of keratinocytes might stimulate the secretion of immunosuppressive interleukins such as TNF-α (App89). The wavelength-dependence of the effects of UV radiation on human keratinocytes is only partly known. *In vivo* experiments demonstrated maximal DNA damage in keratinocytes between 296 and 302 nm (Fre89).

5.3.4 *Effects of UV-B on mast cells*

Mast cells contain a large number of granules that serve for storage of various compounds synthesised by the cell. These compounds can be secreted as a result of external stimuli. Mast cells in the dermis play an important role in different hypersensitivity responses in the skin, of both the direct and the delayed type such as contact hypersensitivity.

It has been observed that, in humans, mast cell degranulation is suppressed by exposure to low dose UV-B, applied as light therapy for the treatment of allergy and eczema (Dan86). Large dose UV-B, on the other hand, can stimulate mast cell degranulation. These are probably not direct effects, since only a small portion of the

UV-B penetrates into the dermis (see figure 6.2), but may be partly mediated by locally produced compounds such as interleukins or prostaglandins.

5.3.5 *Other factors important in UV-B effects*

Suppressor T-cells probably play an important role in the immunosuppressive effect of UV-B radiation. After exposure to UV-B, there was found a relative increase of lymphocytes with cytotoxic activity that considered to belong to the T-suppressor cell population. These cells can suppress the proliferation of lymphocytes and the activity of NK cells. Soluble compounds produced by suppressor cells probably also play a role in this process (Yee89). Apart from local effects in the skin these factors might also have consequences for the systemic suppression of the immune system.

Animals experiments showed that administration of T-4 endonuclease V, an enzyme stimulating the repair of DNA damage, partly inhibits immunosuppression (Kri92). The authors conclude that UV-B-induced DNA damage also plays a role in the stimulation of immunosuppression.

Another possible mechanism of immunosuppression by UV radiation is through the mediator function of *cis*-urocanic acid (DeF83). UV-B can convert *trans*-urocanic acid to the *cis*-isomer. The action spectrum of suppression of contact hypersensitivity in the skin corresponds to that of UV-B-absorption by *trans*-urocanic acid (Noo92). The action spectrum of the photo-isomerisation of *trans*- to *cis*-urocanic acid is different, however (figure 5.3) (Gib93). Suppression of delayed-type hypersensitivity responses to *Herpes simplex* by UV-irradiated urocanic acid has been demonstrated in mice (Ros88). The secretion of TNF-a by keratinocytes, which has been shown to lead to immunosuppression, can also be induced by urocanic acid (Kur92).

5.3.6 *UV-B-induced immunosuppression: summary*

There are several parallel operating mechanisms that result in immunosuppression by UV-B radiation. The influence of UV radiation on Langerhans cells in the skin leads to a decreased antigen presentation by these cells. The number of these cells in the skin also decreases. This results in a locally decreased defense. This is amplified by the induction of T-lymphocytes with suppressor activity (Baa90). In addition to this, the systemic immune system is influenced, since the altered Langerhans cells migrate to the draining lymph nodes and can change the recirculation pattern of T-lymphocytes. Furthermore UV radiation can result in direct effects on lymphocytes, possibly resulting in functional alterations. UV-irradiation of keratinocytes results in secretion of several interleukins that are capable of locally influencing the immune system. If these interleukins end up in the circulation, systemic effects may occur. Finally chemical

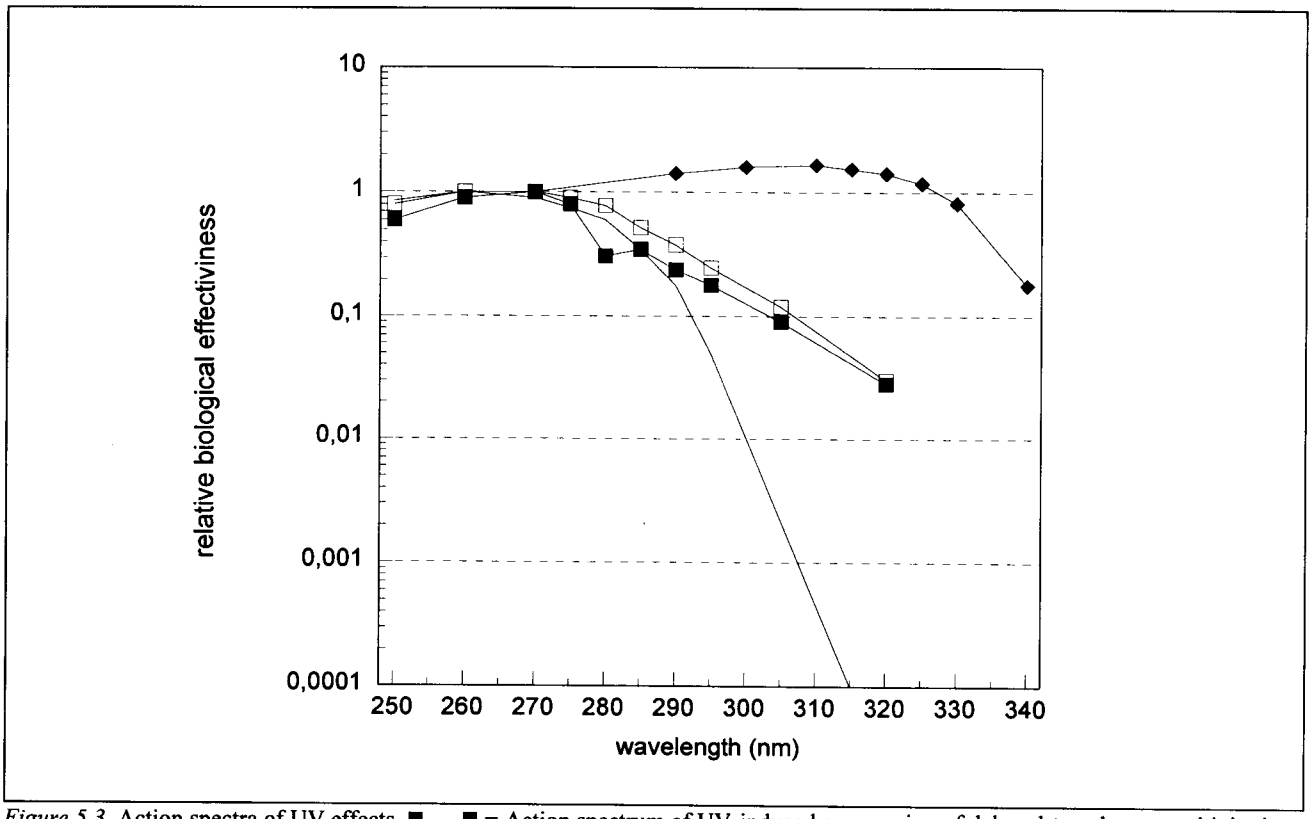

Figure 5.3 Action spectra of UV effects. ■ — ■ = Action spectrum of UV-induced suppression of delayed-type hypersensitivity in mice; □—□ = absorption spectrum of urocanic acid; ◆—◆= action spectrum of the *trans* to *cis* photo-isomerisation of urocanic acid; ——— = DNA action spectrum (Source: Noo92).

mediators originating under the influence of UV irradiation, such as oxygen radicals and *cis*-urocanic acid, may have effects on both local and systemic antigen presentation.

Although there have been some studies into the spectral dependence of UV radiation-induced effects on the immune system (figure 5.3) further research is highly desirable.

5.4 Effects of UV-B on the resistance against tumours

An effect of immunosuppression might be that cells with (photo)antigenic properties (e.g. tumour cells) are no longer destroyed by cytotoxic T-cells. Tumours induced in murine skin after exposure to UV-B are antigenic (Kri74). Such tumours are rejected on transplantation in normal mice, but not when transplanted in UV-B-exposed or athymic mice. This means that T-cell-dependent immunity plays a role in the growth of these tumours and in the influence of UV-B irradiation of this growth.

Melanomas often are also antigenic tumours. Immunosuppression by UV-B might therefore also influence the growth of this type of tumours (Don91). An increase in the incidence of spontaneous leukaemias after exposure to UV-B has been observed in mice (Kri90). Finally the resistance against antigenic tumours induced by carcinogenic chemicals such as benzo[a]pyrene, can also be influenced (Gen91).

Data obtained from patients bearing skin tumours that seem to be induced by UV radiation suggest that the immunosuppressive effect of UV-B in humans is comparable to that in mice (Mor89). Skin exposure to UV-B, followed by application of a contact allergen, led to a reduction of the contact hypersensitivity response in 40% of a group of healthy individuals. This was the case in 90% of a group of patients bearing sunlight-induced skin tumours. Healthy individuals not showing a response could be sensitised to the contact allergen. This was not possible in the skin tumour patients, suggesting the induction of antigen-specific suppression in this group. It has to be established, however, whether this is a direct consequence of UV-B-irradiation or of the mere presence of tumour tissue, that itself also can lead to immunosuppression.

It seems likely that the effects of UV radiation on the immune system contribute to a reduction in the ability to remove transformed cells. The increased incidence of tumours that has been epidemiologically established to be associated with exposure to UV radiation might partly be the result of immunosuppression by UV.

5.5 Effects of UV on resistance against infections

Ultraviolet radiation can lead to activation of latent viruses. This can be a direct effect of UV radiation on the virus and does not necessarily have to be mediated by the immune system. It can be expected, however, that a reduced function of the immune system will have an effect on the resistance against infections. In section 5.2.5 it was argued that, in patients with an immune system suppressed because of an organ transplantation, there seems to be an increased incidence of wart-like skin lesions. This might result from a reduced resistance against infections with human papilloma virus.

In HIV-infected persons, exposure to UV radiation might reinforce the immunosuppression that is already present. Activation of virus replication has also been observed. As mentioned in section 5.2.5, wart-like skin lesions are among the symptoms first to occur in AIDS patients. HIV seropositive individuals are therefore at increased risk of UV exposure-induced adverse effects. In several countries HIV-positive people are therefore advised to avoid direct sunlight.

Exposure to high doses of UV-B (for instance during skiing) can lead to reactivation of latent *Herpes simplex* virus. This indicates the induction of immunosuppression in the skin, allowing a virus to become virulent (Whe75).

Skin lesions resulting from infection with *Leishmania*, a protozoan parasite, are also influenced by UV radiation (Gia86). They appear predominantly on the skin with the highest sunlight exposure. It is remarkable that in mice the severity of these skin lesions can be reduced by exposure to UV-B. An explanation is that the partly immunologically determined inflammatory reaction in the skin is suppressed by UV-B. A severely decreased cellular immune response to *Leishmania* has been observed in mice, however, and the immunological memory for this parasite is also deficient. Antibody production did not decrease after exposure.

Pathological and epidemiological studies of patients suffering from infection with *Mycobacterium leprae*, that causes leprosy, suggest that an increased UV-B exposure may lead to an increased incidence and severity of the lesion (Pat91). In experimental animals, *Mycobacterium tuberculosis*, that is related to *Mycobacterium leprae* in several respects, is used as a model for infection. Both bacteria are a major health problem in tropical and subtropical countries. UV-B exposure of mice preceding and during infection with *Mycobacterium tuberculosis* reduces the delayed-type hypersensitivity reaction to the bacteria. It can be concluded on the basis of other observations also, that the severity of the infection increased.

Candida albicans, a mold that is common in the mouth and on the skin, can cause lesions when there is severe immunosuppression. This may progress into infection of internal organs and the development of a life-threatening situation. Cellular immune responses are very important for resistance against this mold. Exposure to UV-B results in suppression of the delayed-type hypersensitivity response to this pathogen in experimental animals (Den89).

Although it has been established that UV-B radiation affects the course of various infectious diseases no epidemiological data are available on a possible association of the incidence of infectious diseases with exposure to UV-light. An increase in the incidence of infectious diseases has been observed closer to the equator, but several other factors are of importance besides exposure to UV radiation. In this respect it should be noted that the *chance* of an individual being infected depends primarily on the relevant infectious agent but also on the geographical, climatological, cultural, social and economic circumstances. The *reaction* of a person to the infection also depends on a number of factors, e.g. genetic background, age, sex, hormonal status, health status, use of medication, lifestyle and immune status. In a comparison of North-western Europe with Australia and New Zealand, areas that are located at approximately comparable latitudes and that are also rather similar concerning other 'confounders', no apparent differences appear in the incidence and severity of infections, although people living in Australia and New Zealand are exposed to much more UV radiation than inhabitants of North-western Europe. It is not possible, however, to draw conclusions from these observations concerning the effects of UV radiation on infectious diseases.

5.6 Clinical relevance of UV radiation-reduced resistance

UV radiation-induced immunosuppression can be used positively as part of therapy, e.g. light therapy, and in the future, in the prevention of transfusion reactions. There will only be reason for concern when these effects are not wanted and not controllable.

The fact that 90% of skin cancer patients are sensitive to the immunosuppressive effect of sunlight indicates that this effect might play a role in the development of skin tumours. On the other hand, the skin cancer incidence in *psoriasis* patients receiving UV-B light therapy is not or only minimally increased. This might indicate that the role of UV-induced immunosuppression in the induction of cancer is not a major one. However, only limited and fragmented data are available. Patients receiving light therapy also do not show a clear increase in the incidence of infections, but again only limited data are available.

It is likely that the adverse effects of immunosuppression caused by exposure to UV radiation will appear specially in people with an already compromised defense system. UV-induced immunosuppression in the skin might create conditions suitable for a higher risk of development of skin cancer and viral infections of the skin, such as those mentioned above, i. e. human papillomavirus and *Herpes simplex*.

The number of individuals with a compromised immune system - AIDS patients, people with congenital immune defects and patients receiving long-term immunosuppressive treatment after an organ transplant or for other reasons - will increase in the future. Multiple skin tumours in these patients are a major clinical problem that will increasingly make demands of the health care system. Such tumours also show a more malignant course and are associated with higher mortality in individuals with a compromised immune system.

Another possible effect of UV-induced antigen-specific suppression might be a reduced effectiveness of vaccination. This is of importance for the healthy population. Such an effect is most likely to occur in persons that are exposed to a high dose of UV radiation shortly before or during immunisation. The effect of UV radiation on the Langerhans cells, that are responsible for the induction of the primary immune response, is of prime importance. Once induced, immunity is generally difficult to modulate. In this case, the relatively modest UV-induced immunosuppression will not have important consequences for immunity. Local effects of UV-B, however, can be harmful even after induction of immunity.

Finally, derangement of the immune system by UV-B radiation may have negative consequences for autoimmunity. It is known that UV light can induce skin lesions in patients with the auto-immune disease, *lupus erythematodes*. In fact, this phenomenon is used in the clinic as a diagnostic criterion. Almost no research has been done into

the aggravation of autoimmunity by sunlight, but it is known that autoimmunity is often accompanied by immunodeficiency.

5.7 Protection against photo-immunological effects

Only few data are available regarding protection against immunological effects of exposure to UV radiation. Experiments with pigmented test persons showed that pigment does not offer protection against induction of immunosuppression by UV radiation. Most sunscreens that protect against UV-B radiation-induced DNA damage do not provide adequate protection against immunosuppression. Some protection by sunscreens against the suppression of certain lymphocyte functions has been found *in vitro*. The SPF factor of sunscreens, which indicates the protection against sunburn, is according to this information of no predictive value for immunosuppression. *In vivo*, these sunscreen offer almost no protection, if any, against inhibition of certain lymphocyte functions. An exception perhaps are the cinnamate-containing sunscreens (Wol93a). Recently it has been suggested that sunscreens might offer 100% protection against the induction by sunlight of *Herpes labialis* (Roo91).

Problems that need to be solved in this respect are, for instance, what are the pharmacokinetics and the toxicity of sunscreens? at what level of the skin do they offer protection? in what way do they interfere with mediators for UV-damage in the skin and what is their influence on the dose- and time-dependence of UV radiation-induced immunosuppression?

5.8 Therapeutic application of UV radiation

In some cases suppression of the immune system by UV radiation can be beneficial. This is used in the treatment of certain skin disorders such as eczema and *psoriasis vulgaris*.

5.9 Conclusions and recommendations

Animal experiments show that UV radiation can induce biologically significant effects on the immune system and on resistance against infections. Effects on immunological parameters in humans are also known. It has to be noted, however, that, although the immune system in humans and animals is grossly comparable, extrapolation of experimental data obtained in animals to the human situation is difficult. This is also the case for the effects of UV radiation. Animals usually have a furry skin that will also not be highly exposed to sunlight because they are twilight or nocturnal animals. The sensitivities of the human and animal skin can be different, and therefore so can the

sensitivity of the immunologically relevant components. The mechanisms of protection against certain infections and the reserve capacity of these mechanisms may be different in humans and animals. Therefore it is possible that effects observed in animals may lead to overestimation, but also to underestimation, of the severity of the effects in humans. This is the case both for infectious diseases and for tumour growth.

Experimental and epidemiological data show that there is a relation between skin tumour incidence and exposure to sunlight (see chapter 6). It is not known, however, to what extent an effect of UV radiation on the immune system is involved in the development of human skin tumours.

It is likely that increased exposure to UV-B radiation, resulting from depletion of the ozone layer, will have an influence on the incidence of infections and auto-immune diseases, and possibly also on that of tumours, through effects on the immune system. The information presented here shows clearly, however, that the data are still insufficient to allow quantitative estimations of the risk involved. More research is necessary before such estimations can be made.

First, epidemiological studies have to be performed that are aimed at comparison of different regions and a coupling of meteorological data with the recording of infectious diseases, tumours, auto-immune diseases and the efficiency of vaccination. Also the question of tourism to sunny areas must be considered. Special attention has to be paid to age-dependence: young children and older people are at increased risk of infectious diseases and, because of this, possibly also of a reduction in the resistance to infectious diseases that is associated with exposure to UV-B radiation.

Cohort studies might be performed in patients that receive (long-term) light therapy, HIV-positive people, patients suffering from autoimmune diseases and transplant patients. Also, the effects on basal immunological parameters might be studied in volunteers.

Animal experiments might be done to study the spectral- and dose-dependence of the effects of UV radiation on basal immunological parameters, as well as the effects on resistance against systemic infections and effects on auto-immunity. Also, experimental studies should devote more attention to the effects of vaccination.

This research might lead, not only to an estimation of the risks of the effects of UV exposure to the immune system, but also to a better definition of groups at risk.

In addition to more studies being done it should be recommended, even at this early stage, that persons that can be expected to belong to groups at risk be advised to abstain from excessive sunlight exposure.

Chapter 6

Skin effects

After a short introductory description of on the structure of the skin, the committee summarises in this chapter the most important aspects of carcinogenesis and describes a number of skin disorders that can be caused by sunlight. Attention is directed to the location on the body surface where these lesions may arise and to certain groups of patients for whom the risk of skin disorders is increased. This is followed by an elaborate description of skin cancer epidemiology, in which the committee investigates to what extent exposure to sunlight plays a causative role in the development of the different forms of skin cancer. In the last part of this chapter the chance of the development of skin cancer as a result of sunlight exposure is quantified as realistically as possible, being based on the results of experimental and epidemiological research.

The economic consequences of skin cancer are not dealt with in this chapter. The committee feels it not truly possible to determine these because of the presence of several uncertain and complicating factors, such as the costs of lost years and the lowered quality of life. The committee does, however, want to point out that, for Australia, the total direct medical costs for melanomas and other skin tumours are estimated at AU$ 400 million annually (McC89). The costs for melanomas carry a higher estimate than those for other skin tumours, mainly because melanomas develop at a relatively young age and have a bad prognosis. Failure to recognise a melanoma at an early stage may lead to death, and these cases concern mainly people in their economically productive period of life (Mar89, McC89).

6.1 The skin

The skin is the organ especially exposed to external factors such as ultraviolet light. The skin consists of two layers: the epidermis and the dermis (figure 6.1A). The two are separated by a basal membrane (figure 6.1B).

6.1.1 The epidermis

The epidermis is the outer layer of the skin and therefore the most UV radiation-exposed part of this organ.

The constituents of the epidermis (figure 6.1B) are the keratinocytes (basal and squamous cells) which form a tight barrier. Interspersed between these keratinocytes are dendritic cells (mainly melanocytes and Langerhans cells). The keratinocytes are able to proliferate, differentiate and move to the outer cell layers. During differentiation keratin is formed in the cells and finally only dead cell bodies remain that are completely filled with this substance. This process is called keratinisation. In addition to keratin, several substances are produced by the keratinocytes that play a role in the immune system, for instance the cytokines interleukin-1 and TNF-α, and adhesion molecules. Keratinocytes therefore can also be actively involved in immunological processes. The Langerhans cells are especially dedicated immune cells. Their primary function is presentation of antigen (see chapter 5). They are capable of migrating from the epidermis through the basal membrane and are then transported via the lymphatics to the lymph nodes.

The formation of pigment in the epidermis occurs in the melanocytes. The process is stimulated by sunlight. The two kinds of melanin that are formed, pheomelanin and eumelanin, were mentioned in section 4.4. Both kinds are stored in specific organelles, the melanosomes. These can subsequently be taken up by the keratinocytes. Eumelanin is supposed to offer protection against UV radiation, while pheomelanin can be damaging on UV irradiation (see section 4.4).

These cell types form the vast majority of cells in the epidermis. Other cell types are not discussed here.

6.1.2 Dermis

The dermis contains fibroblasts that form collagen and elastin fibers. These compounds are excreted and form an extracellular supporting tissue. Also present in the dermis is a network of blood capillaries, a system of peripheral nerve endings, the adnexes (hair follicles and sebaceous glands) formed by epidermal cells and the sweat

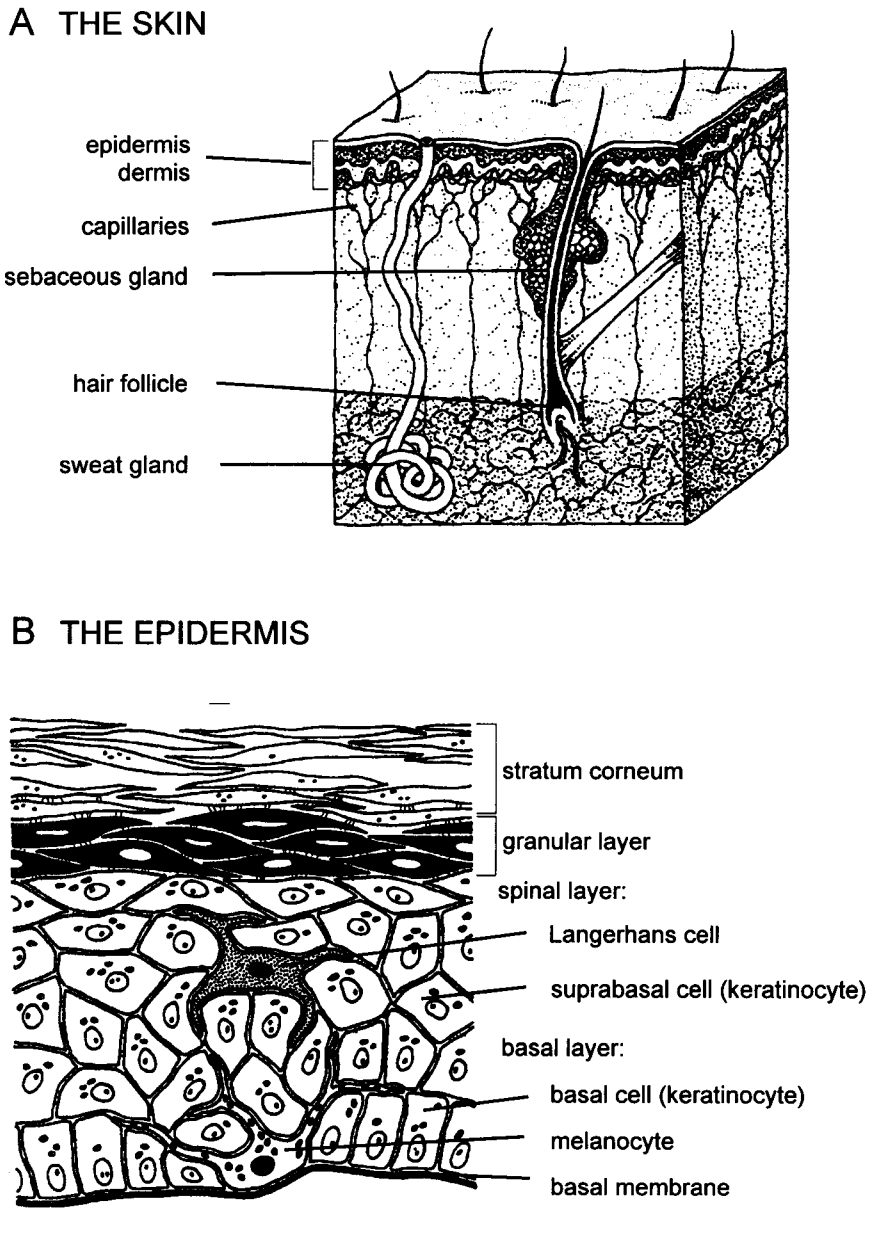

Figure 6.1 Schematic overview of the structure of the skin (Source: Smi82) and of the epidermis (Source: Vin93).

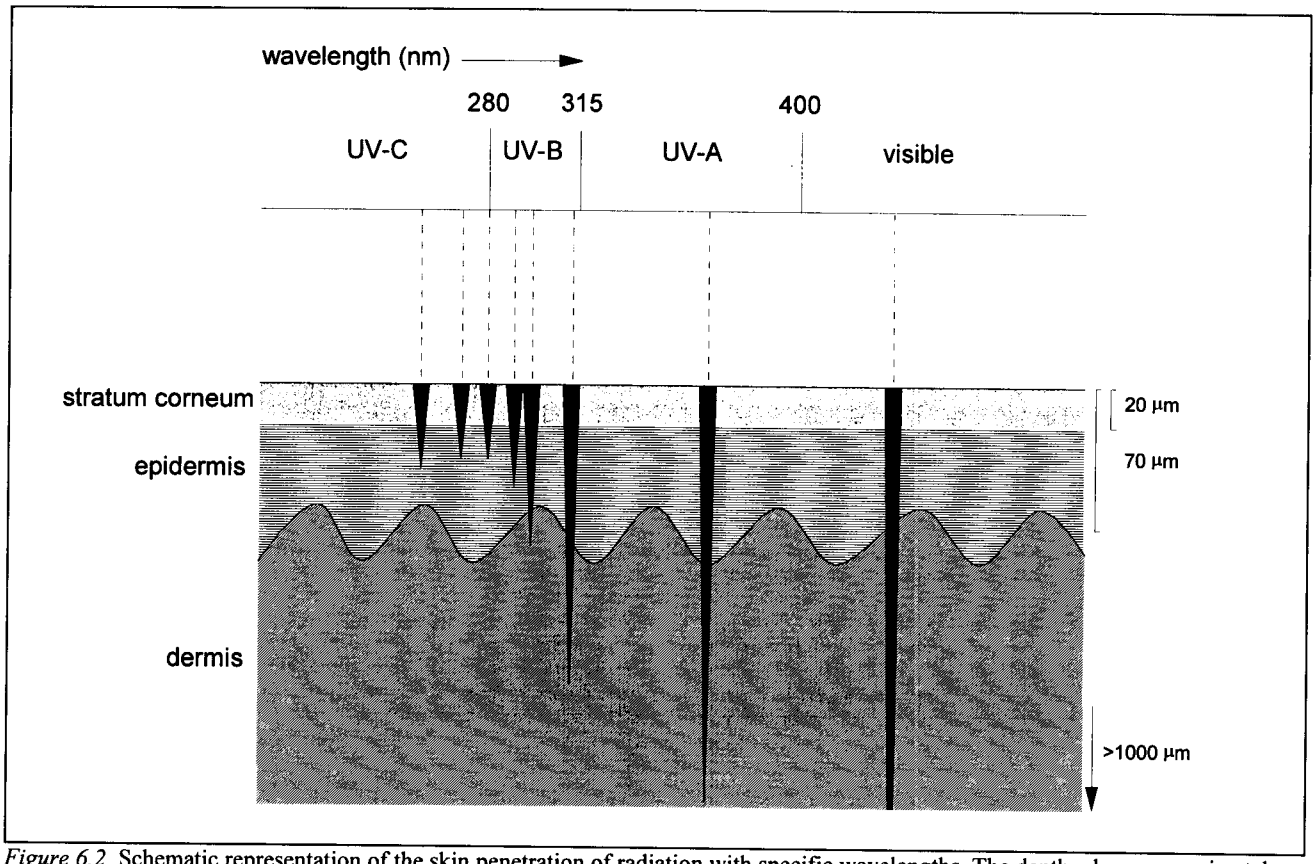

Figure 6.2 Schematic representation of the skin penetration of radiation with specific wavelengths. The depth where approximately 1% of the initial energy remains is shown. Only the upper part of the dermis, the total thickness of which can be up to several mm, is shown (Source: Bru84).

glands leading to the skin surface through the epidermis (figure 6.1).

Under normal conditions the border between the epidermis and the dermis has an undulating aspect. Thus, the dermis has papillary extrusions that reach rather close to the skin surface. These dermal papillae contain a capillary network. In view of the penetration of the different wavelengths of UV light, UV-B will be able to reach not only the epidermis but also part of the dermis, particularly the blood vessels located in the tip of the dermal papillae (figure 6.2).

6.1.3 *UV-absorbing compounds*

The stratum corneum, formed by the dead cornified epidermal cells, contains keratins. Urocanic acid is formed in the upper layer of the epidermis. All these compounds are capable of absorbing UV light. Deeper down in the epidermis UV can be absorbed by pigments (melanins), DNA and proteins in the epidermal cells.

6.2 Sunlight-induced carcinogenesis

In this section, before starting with a description of the different skin cancers, the committee presents a short introduction to the process of carcinogenesis.

6.2.1 Mutation, transformation and repair of damage

Every form of cancer is the result of inheritable changes, mutations, in cells. A cell that has collected a sufficient number of certain mutations can transform and eventually grow out to become a tumour. The genes in which these inheritable changes occur, and that are therefore involved in the development of cancer, are the oncogenes and the tumor-suppressor genes. Expression of an oncogene promotes the development of a tumour, expression of a tumor-suppressor gene inhibits it (Bis91, Wei91a). The number of oncogenes and tumor-suppressor genes involved in carcinogenesis is not known exactly, but varies with the type of tumour and the tissue in which the tumour develops. There are at least three, but probably more than six genes involved in this process (Fea90, Nak94). Damage to the DNA resulting from exposure to carcinogens or radiation (both ionising and UV radiation) increases the chance of inheritable changes in these genes. This chance is greater in tissues with proliferating cells than in tissues with resting cells. Therefore stimulation of cellular growth has a stimulating effect on the development of tumours.

It is also to be expected that the risk of developing a cancer is enhanced when one or more genes involved in tumour formation mutated in early childhood. In this case the process was initiated early in life and there is a longer time period available for the development of new inheritable changes in the other genes involved in tumourigenesis. Therefore, limitation of DNA damage early in life, e.g. by limiting exposure to UV radiation, can substantially decrease the risk of tumour development.

Generally, cells posses effective mechanisms to remove damage from their DNA. In mammalian cells nucleotide-excision repair is the most important mechanism for the removal of cyclobutane pyrimidine dimers and pyrimidine(6-4)pyrimidon photoproducts (see section 4.3). This is not done equally effectively in all parts of the DNA (Mul91). The DNA of genes that are permanently used in the cell, the so-called housekeeping genes, is repaired considerably faster than DNA sequences that are not expressed. Moreover, the transcribed DNA strand, i.e. the strand used for the synthesis of messenger RNA (mRNA), appears to be repaired faster than the non-transcribed strand. It seems that all this is aimed at repairing as quickly as possible those parts of the DNA that are essential for functioning of the cell.

One of the best characterised examples of the relation between exposure to a DNA-damaging agent and the development of tumours, and the importance of repair of DNA damage, is found in the inheritable disorder, *xeroderma pigmentosum* (XP). The skin of XP patients is extremely sensitive to sunlight and the incidence of skin cancer in these patients is increased 1000-fold compared to the normal population. The cause of this disease appears to be a malfunctioning DNA repair mechanism. UV-induced DNA damage is not or not properly repaired in cells of most XP patients, as a result of a deficiency of nucleotide-excision repair (see section 4.4). In some cases the deficiency involves primarily the repair of non-active genes while in other cases there is a general decrease of the level of nucleotide-excision repair (Ven91).

6.2.2 *DNA damage and DNA replication*

An important step in the reaction of a cell to damage of the DNA is inhibition of the DNA synthesis that precedes cell division, i.e., replicative DNA synthesis (Pai80, Sme79, Wei88). Most likely this reaction offers the cell time for optimal repair of DNA damage, because if the damage has not been repaired before the onset of DNA replication, it can result in faults in the new DNA that can then contribute to cellular transformation (Coh91). Inhibition of replicative DNA synthesis is an important mechanism for reducing the risk of cellular transformation.

6.2.3 *Mutations of oncogenes and tumor-suppressor genes*

The proliferation of normal cells is partly regulated by oncogenes and tumor-suppressor genes coding for proteins that are, respectively, growth-promoting and growth-suppressing (Wei91a). Alterations in these proteins can cause uncontrolled cell growth. Recent data indicate that the product of the p53 gene, a tumor-suppressor gene, can inhibit DNA synthesis (Dil90, Kas91, Mar91). Changes in this gene could contribute to the development of a tumour. Mutations in the p53 gene have been found in many human tumours, also in tumours that might have been induced by UV radiation, such as basal cell carcinomas, melanomas, *keratosis actinica* and squamous cell carcinomas (Bra91, Rad92). Mutations in the p53 gene have also been found in squamous cell carcinomas induced in hairless mice by UV radiation (Kre92).

An investigation of melanomas showed that all primary tumours located on skin parts that are generally heavily exposed to sunlight contained mutations in the *N-ras* gene, a proto-oncogene (Vee89). These mutations were all located next to or in the direct vicinity of a dipyrimidine sequence (TT or CC), which is a strong indication of the involvement of UV. Such *N-ras* mutations have also been found in melanoma cells of XP patients (Kei89) and in human basal and squamous cell carcinomas (Sch90).

Disturbance of cellular growth regulation leading to the development of cancer is not the exclusive result of genetic changes in growth-regulating genes. There are also indications that alterations in the patterns of expression of these genes appear without changes in their base sequence (the so-called epigenetic changes). Possibly connected with this is the induction of 'stress' processes by UV exposure of cells, e.g. the formation of so-called 'stress' proteins, that make cells more resistant to the consequences of a subsequent UV exposure or to other genotoxic agents.

6.2.4 *Photo-immunology*

Results of recent investigations indicate that the immune system plays an important role in UV-induced carcinogenesis. This was described in detail in chapter 5.

6.2.5 *Conclusion*

Mutations in oncogenes and tumor-suppressor genes, like *ras* and p53, seem to play an important role in the development of tumours affected by UV radiation. These mutations may arise as a result of the presence of a dipyrimidine photoproduct. The consequence may be that the regulation of the cell cycle, important in the proliferation of cells, is defective. This may finally lead to the development of tumour cells. Skin exposure can also lead to suppression of the immune system, which may result in inadequate removal of tumour cells. Finally, irradiation of the skin with UV light may stimulate cellular proliferation, which can promote the growth of mutated cells.

6.3 Clinical concepts

In this section the committee presents a short description of the various skin disorders that are connected with exposure to UV radiation. These skin disorders are divided into those arising from the keratinocytes and those arising from the pigment-producing cells, the melanocytes.

6.3.1 *Non-melanocytic skin cancer (NMSC)*

Actinic keratosis (= keratosis solaris)

Thickening of the epidermis accompanied by abnormal keratinisation. This condition is often found in skin heavily exposed to sunlight.

In addition to the thickened keratin layer the epidermis contains atypical keratinocytes with disturbed keratinisation (dyskeratosis).

Morbus Bowen

When the number of cells with a keratinisation disorder and atypical characteristics is high, the lesion is called an intra-epithelial carcinoma: *M. Bowen.*

Squamous cell carcinoma (= carcinoma planocellulare) (SCC)

When there is a further increase in the number of abnormally keratinised cells and when there is an abnormally high number of cell divisions and when such abnormal cells penetrate the basal membrane, the lesion is called a squamous cell carcinoma.

Actinic keratosis , *M. Bowen* and SCC are all characterised by alterations in the differentiated keratinocytes.

Clinically, SCC is characterised as a tumour with abnormal or increased keratinisation. It occurs almost exclusively on skin areas exposed to sunlight. The risk of metastasis is generally very low (2 - 3%), except when SCC is located at the outer rim of the ear, mucous membranes, the lip or the fingers. In these cases the risk of metastasis is increased.

Actinic keratosis is often considered a precursor of SCC. Recent research has demonstrated, however, that only a small number of these lesions develop into an SCC. Therefore it is better to consider actinic keratosis as a lesion indicating a pathologically altered skin area, in which the risk of development of an SCC is increased (Mar88a, Pre92).

All lesions described so far can arise as a result of exposure to UV radiation. They are also found, however, in persons exposed to toxic compounds, such as arsenic or tar. This indicates that carcinogenic or co-carcinogenic factors other than exposure to UV light may also play a role (Pot75).

Basal cell carcinoma (= carcinoma basocellulare) (BCC)

These tumours develop from proliferation of keratinocytes present in the epidermis or hair follicles. They are less malignant and hardly tend to metastasise. Clinically these carcinomas are characterised by a translucent or pearly appearance and several well-visible blood vessels (telangiectasis).

In the English language literature NMSC usually includes *M. Bowen*, SCC and BCC. They are, however, different types of skin lesions.

6.3.2 *Cutaneous malignant melanoma (CMM)*

Skin melanomas are highly malignant tumours. When they have reached a certain thickness they can readily form metastases. The prognosis therefore is strongly determined by the thickness of the tumour and the connected infiltration into the dermis.

When melanomas are detected before they reach a critical thickness (approximately 1 mm) life expectancy is still favourable.

Naevus naevocellularis

A brown to light-brown coloured benign tumour of variable size, histologically characterised by pigment-producing cells grouped in nests in the dermis or in the dermis and epidermis.

Clinical atypical naevi

These pigmented skin tumours are characterised by a somewhat serrated border and irregular pigmentation. The brownish naevus is often located on a reddish background. Histological examination reveals naevus cells with a sometimes polymorphic character. Some fibrosis is often present around the naevus cell nests and there is an inflammatory infiltrate. In principle these atypical naevi are benign. Complaints of itch or alterations in pigmentation can be indications of malignancy.

Lentigo maligna

This lesion is also called Hutchinson melanotic freckle. It is a highly irregularly pigmented flat lesion with serrated borders. *Lentigo maligna* is almost exclusively found on the face or the lower arms, especially in people who have been exposed to sunlight for a prolonged period of time. Histological examination often shows a sunlight-altered skin that contains an increased number of melanocytes. Some melanocytes have a polymorphic nucleus. These lesions are considered pre-cancerous.

Lentigo maligna melanoma (LMM)

Compared with *lentigo maligna* the variation in colour in these skin lesions has increased. LMM also rises above the skin surface. The number of atypical melanocytes is increased and these can also infiltrate into the dermis.

Melanoma in situ

This is a lesion of atypical melanocytes located exclusively in the epidermis.

Superficial spreading melanoma (SSM)

This is the most common type of skin melanoma. This skin tumour too is irregularly bordered and pigmented. SSM can slowly grow horizontally in the skin for many years before commencing a prognostically unfavourable growth into deeper skin layers. A histological characteristic is the presence of polymorphic nuclei and atypical melanocytes that migrate to the surrounding epidermis. Some infiltration of atypical melanocytes into the dermis is also found.

Nodular melanoma (NM)

The nodular melanoma is an inconsistently pigmented and sometimes even unpigmented tumour. It consists of atypical melanocytes that infiltrate in the dermis rather early.

Acrolentiginous melanoma (ALM)

By definition this melanoma is located on the acra: palms, soles, under the nails and also on the lip. It is the most common melanoma in pigmented races.

6.4 High-risk groups for UV carcinogenesis

There are various groups of patients at increased risk of the development of skin cancer. Exposure to UV light appears to plays an important role. Specific research into these diseases should provide important information on the development of skin cancer. This concerns the following (rare) diseases.

Xeroderma pigmentosum (XP)

This is an inheritable disease with a defect of nucleotide-excision repair. It was dealt with in the section on UV carcinogenesis (section 6.2.1).

XP patients need to avoid sunlight from very early childhood on. Their life expectancy is limited and they mostly die before the age of 20 years from the consequences of NMSC and CMM (Kra87a).

Albinism, Rothmund Thomson's syndrome and basal-naevus syndrome

These are genetically determined diseases. The patients are at increased risk of the development of skin cancer from UV radiation (Ber87). Albinism holds an increased risk of both CMM and NMSC; Rothmund Thomson's syndrome and basal-naevus syndrome are associated only with an increased risk of development of NMSC.

Familial atypical multiple mole melanoma (FAMMM)

FAMMM patients have a genetically determined increased risk of development of malignant melanoma. Exposure to UV radiation seems to be able to further increase this risk. The recent localisation in FAMMM patients of the responsible gene will lead to a further classification of these patients (Can92, Gru93a).

People suffering from FAMMM are also at increased risk of melanoma of the eye and pancreatic cancer.

Reduced resistance

Patients with a compromised immune system are also at increased risk of development of NMSC and CMM (Bou92, Har90). This population has been dealt with in chapter 5. Here we merely conclude that organ transplantations and the increasing possibilities of supporting AIDS patients will result in an increased number of patients with defective resistance.

6.5 Sunlight-induced skin ageing

Sunlight-induced skin ageing can readily be distinguished from intrinsic ageing processes (Bal89). Clinical manifestations of the ageing processes in the skin under the influence of sunlight are the formation of wrinkles, a thickened, yellowish skin and spotty pigmentation. Actinic keratosis might also be counted among these ageing phenomena. It has been histologically established that, with sunlight-induced ageing, there is initially an increase in the number of cells in the epidermis and in the mass of the dermal matrix (elastin fibers, basal substance and collagen). UV-induced changes are also found in the dermal blood vessels, e.g. dilatation of the vessel wall. Animal experiments allow UV-induced dermal changes to be further quantified. Exposure of mice to UV radiation resulted in elastosis. Irradiation with a UV-A dose of 3000 $J \cdot cm^{-2}$ had the same effect as a UV-B dose of 5 $J \cdot cm^{-2}$ (Kli85). It is possible that, besides a

direct effect of UV radiation on the dermal extracellular matrix, certain mediators are mobilised which subsequently have an effect on the composition of this matrix.

6.6 Other skin disorders

The other skin disorders that may be provoked by UV radiation, for example polymorphic light eruption, *lupus erythematodes* or phototoxic reactions when using drugs, are only briefly mentioned here.

Polymorphic light eruptions, reactions of the skin following sunlight exposure, are the most common of these conditions. Many people exhibit such reactions in early spring as a result of the loss of adaptation of the skin to sunlight during the dark winter period. During spring and summer, adaptation is restored and the reactions disappear. A small number of individuals exhibit a persisting reaction that might even still be present in fall and winter.

The wavelengths for which there is hypersensitivity can be both in the UV-B and UV-A range. A change in the UV spectrum resulting from depletion of the ozone layer probably will not have a clear effect on the prevalence of polymorphic light eruptions and persisting sunlight hypersensitivity. Extra UV-B in the dark period can even be beneficial for these patients. Regular exposure can prevent the loss of adaptation of the skin (Leu93).

When using drugs one has to be on the alert for possible phototoxic or photoallergic reactions. These can be caused by interaction of long-wave UV radiation and the drug or a metabolite.

The UV action spectrum for the development or aggravation of *lupus erythematodes* is not known.

6.7 Pigment

Skin cancer develops at a much lower frequency in people with a negroid skin, despite the fact that they often live in countries with high sunlight radiation levels (Yoh92). The intrinsic sensitivity to the induction of DNA damage by UV radiation is the same in melanocytes and keratinocytes. The relative large number of melanocytes in the skin of pigmented races is therefore not the reason for the difference in tumour incidence in less pigmented races.

It is suspected that pigment, and primarily eumelanin, protects against the development of skin malignancies. Eumelanin is present in high concentrations in the skin of highly pigmented persons. These people present with skin tumours primarily in the less pigmented skin areas (for instance acrolentiginous melanomas, see section 6.3.2).

Another indication of a possible protective action of eumelanin is the observation that red-haired people, who have relatively more pheomelanin, have a higher incidence of skin cancer, both NMSC and CMM. Pheomelanin is also present in higher concentrations in atypical naevi, known as melanoma precursors (see section 6.3.2). UV irradiation of pheomelanin is more likely to result in the formation of oxygen radicals than in protective effects. This is especially the case when pheomelanin is not located in the melanosome but occurs free in the cytoplasm (Har80).

In order to interpret the data on the association between sunlight exposure, development of skin cancer and pigmentation the pheo-/eumelanin ratio and the stability of the melanosomes needs to be accounted for. It seems likely that the difference in metabolism of pheo- and eumelanin plays a role in the development of melanomas from (atypical) precursor lesions.

6.8 Epidemiological data

In the interpretation of epidemiological data it is necessary to take into account a number of criteria that have to be met by sound epidemiological research. One of the most important is that confounding factors have to be reckoned with or corrected for. The committee has dealt with this in a general sense in chapter 3. As an introduction to the discussion of several recent epidemiological studies into the relation between sunlight exposure and the development of skin lesions, the committee singles out several confounding factors that are specific to these studies.

First it has to be realised that the reaction of the skin to exposure to sunlight depends on its sensitivity to sunburn and on its capability to tan (Ste84). On the basis of these criteria different skin types are distinguished:

- *type 1*: always burn, never tan
- *type 2*: often burn, hardly tan
- *type 3*: seldom burn, always tan
- *type 4*: never burn, fast and easy tan

As people with skin types 1 and 2 sunburn more easily than people with types 3 and 4, they will generally expose themselves to sunlight for shorter periods of time. It is therefore important for correct interpretation of the epidemiological studies that information on skin type and on episodes of sunburn also be taken into account (Arm88).

Epidemiological studies often present incidence data. It is important to know whether incidence data for skin cancer are based exclusively on the first-occurring tumour or whether later occurrences at different body locations are also counted. Although they only constitute a small group, there are patients who develop more than

one skin tumour in a relatively short period of time. These multiple skin tumours are not always included in the incidence. It is also important whether *Morbus Bowen*, an intraepithelial carcinoma, is included in the determination of SCC incidence.

In screening or prospective studies the skin is generally carefully examined. More skin malignancies (including so-called sub-clinical lesions) will therefore be found in such studies than in retrospective ones.

6.8.1 *Epidemiology of NMSC*

NMSC is the type of skin cancer for which induction by UV radiation has been most convincingly demonstrated in both experimental and epidemiological studies. Sufficient data are now available to allow a quantitative estimation of the relation between exposure and incidence. This is done in section 6.9. In the present section the committee gives an overview of the most important epidemiological studies published after the appearance of the 1986 Health Council report on risks of UV radiation (GR86). The results of these studies will be used to further support the hypothesis of a causal relationship between sunlight exposure and the development of NMSC.

NMSC is found almost exclusively on sun-exposed skin areas, primarily in caucasians (Sco81). People exposed to high doses of UV radiation, who hardly tan and are sensitive to sunburn, run the highest risk of skin cancer (Vit80).

The 1986 Health Council report (GR86) is based primarily on the results of a study by Scotto (Sco81). This author determined the NMSC incidence in the caucasian population at different latitudes in the USA for the period 1977-1978. These data are unique in that they were gathered in a specially dedicated survey of relatively comparable populations (US whites) from a wide range of geographical locations; diagnostic and registration procedures were standardised. This study showed that NMSC incidence in the caucasian population is higher in Texas than in more northern states in the USA. The incidence therefore seems to be latitude-dependent.

Similar epidemiological studies cannot be performed in Europe, since the population in Southern Europe predominantly has skin type 3 and that in Northern Europe skin types 1 and 2.

Europe and USA

Recently Coebergh *et al.* (Coe91) reported on an extensive epidemiological study on NMSC in the south-eastern part of the Netherlands. The incidence of BCC and SCC was determined in a population of 650,000 people over the period 1975 - 1988. An increase in the incidence of BCC was found on sun-exposed skin areas like the head,

neck and trunk. There was also a significant decrease in SCC incidence on the lips. These data are consistent with data from the USA. A study in Portland (USA) determined the incidence of SCC and melanomas in a 300,000+ population (90% caucasian) between 1960 and 1986 (Gla89). Both SCC and melanoma incidence had increased considerably during the study period.

A comment to this study was that the increase in incidence can be partly explained by an improvement in diagnosis (Wei89). Because of this, more skin lesions are now diagnosed as SCC than were previously. It was also pointed out that the mortality due to SCC decreased - despite the increase in SCC incidence. Increased SCC mortality was observed only for white males in the period 1980 - 1988 (Wei91b). Unfortunately no adequate mortality data are available for the Netherlands.

Australia

Recent Australian epidemiological studies have provided a number of indications of an association between sunlight exposure and the development of NMSC. A study among 2095 inhabitants of Queensland retrospectively determined the number of people treated for skin cancer during a two-year period. The calculated NMSC incidence for individuals aged 20 - 69 years was 2389 per 100,000 person-years for males and 1908 per 100,000 person-years for females (Gre90). The incidence of BCC was 4.5 times higher than that of SCC. The presence of skin lesions characteristic of damage by sunlight, for example actinic keratosis and elastosis of the neck, indicated a highly increased risk of BCC and SCC. The population with skin type 1 in Queensland is considered a unique group for the study of the induction of skin cancer by sunlight (Fit92).

Another important study is the one performed with the population of Frankston, a town located 38 kilometers south-east of Melbourne in the southernmost tip of Australia (Mar90). This study included 1232 persons over 40 years of age who were born in Australia and had lived in Frankston for at least 20 years. Also included were 1332 British immigrants living in Frankston. All subjects were examined for the presence of actinic keratosis, a skin lesion that may develop into SCC (see section 6.3.1). The prevalence of actinic keratosis appeared to be significantly higher in people born in Australia. However, in immigrants who had moved to Australia before age 20 the difference disappeared with time (figure 6.3). The authors considered this to be an indication that the prevalence of actinic keratosis and SCC is associated with sunlight exposure during early childhood.

The same investigators determined NMSC incidence in a five-year (1982 - 1986) longitudinal study of 2669 people over 40 years of age from Maryborough, a town located 110 kilometer north of Melbourne (Mar89). The incidence in males aged up to

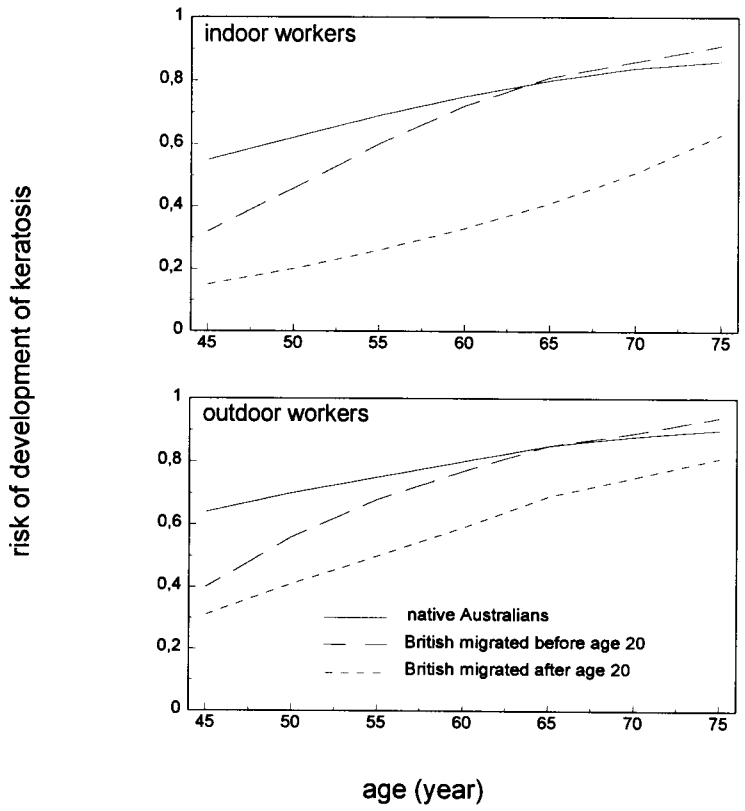

Figure 6.3 The risk of the development of actinic keratosis for different groups of male inhabitants of Frankston, Australia (Source: Mar90).

55 years was not influenced by their having an indoor or outdoor occupation. At ages over 55 years the NMSC incidence showed a greater increase in individuals employed outdoors. This is seen as an indication that the incidence of NMSC before the age of 55 years is primarily determined by sunlight exposure during childhood (McC89). Further support for this inference comes from the recently published high prevalence of NMSC in WW II veterans who had been stationed in the Pacific (Ram93).

A possible explanation for the high risk attached to childhood exposure is twofold. Firstly, the time available for carcinogenesis is longer with childhood exposure than with exposure at later ages. Secondly, it is probable that young people have relatively more proliferating skin cells than do older people. Since the mutagenic effect of UV radiation on dividing cells is considerably greater than on resting cells, the chance of a cell undergoing malignant transformation is also greater.

The committee believes that the relation between accumulated dose of sunlight and SCC has been demonstrated convincingly (Sco81, Vit80, Vit90). The relation between sunlight and BCC is also clear, but not as straightforward as that for SCC. Considering the locations of BCC on the body, most of these tumours (80 - 95%) are caused by sunlight.

Individuals with a suppressed immune system

Kidney transplant patients are under continuous treatment with immunosuppressive drugs and are at increased risk of the development of NMSC and wart-like skin lesions. The risk of development of SCC for these patients in the Netherlands is increased 500-fold. As a result of the absence of a properly functioning immune system the risk of metastasis and mortality is also increased.

In the Netherlands, the first NMSC in these patients are observed 6 - 8 years after transplantation. Sunlight exposure before the age of 30 is a particularly dominant factor for the risk of development of NMSC after kidney transplantation (Bou93a). The faster increase of the cumulative NMSC incidence in kidney transplant patients in Australia supports this conclusion.

(Immuno)genetic factors also appear to play a role in the development of NMSC (Bou93b). It is important to investigate whether such factors generally indicate predisposition to the development of these tumours.

In other individuals with a suppressed immune system, such as AIDS patients, NMSC incidence is increased as well, together with the subsequent risk of metastasis and mortality.

Main conclusions

The main conclusions of the committee are the following:

- The risk of development of NMSC is mainly determined by the amount of sunlight exposure. The epidemiological data of Scotto (Sco81) are still the most suitable material for the quantification of the relation between sunlight exposure and the prevalence of NMSC.
- Recent studies of migrants in Australia indicated that sunlight exposure during childhood (before the age of 20 years) is a greater risk factor for the development of precancerous skin lesions than is exposure at later ages. The explanation is the longer time available for carcinogenesis and an increased risk of mutations resulting from the larger compartment of proliferating (and mutation-sensitive) cells.
- An increase in BCC has been observed in the Netherlands between 1975 and 1988. An increase in SCC was not evident.

- Studies on kidney transplant patients indicate that their highly compromised immune system increases the risk of the development of NMSC. Immunogenetic factors can also contribute to this risk.

6.8.2 *Epidemiology of CMM*

As already mentioned in the introduction the committee limits itself in this chapter to the association between sunlight exposure and the development of skin lesions. The committee that drafted the 1986 report did not consider it possible to deal with the relation between sunlight exposure and the development of CMM, because of lack of adequate data. The results of epidemiological studies published since, especially those performed on immigrants in Australia, indicate that an association between sunlight exposure and (certain types of) CMM is likely. An association also seems to exist for the development of benign tumours (*naevi naevocellularis*). Furthermore a new syndrome has been described, FAMMM (see section 6.4). Indications of a role of sunlight in the development of melanomas are also provided by data from FAMMM patients.

The literature shows that for the development of melanomas the relationship with sunlight exposure is much less clear than it is for NMSC. Considerable differences exist between the different types of CMM (see section 6.3.2) and the exposure pattern also seems to have a major influence. For some melanomas, i.e., SSM and NM, higher incidences have been found on skin area that are not regularly exposed to sunlight, but that may receive a massive dose in a short period of time, for instance during holidays. This has led to the intermittent exposure hypothesis, which states that the pattern of exposure is of more importance than the total radiation dose for these types of CMM. For other CMM types, however, the total dose does seem to be the determining factor, as it is with NMSC. There are also indications that sunlight exposure during early childhood may have a relatively greater influence on the development of certain types of melanoma than exposure at higher ages.

In the section that follows the committee, after presenting a short overview of the incidence data, discusses several selected studies that can be considered to confirm the above-mentioned hypotheses.

Incidence and mortality

The incidence of skin melanomas in the caucasian population has increased dramatically in the past 30 years. This increase is especially detectable in those countries where there has been an adequate cancer registry during this period. The increase in incidence sometimes is as high as 2 or 3-fold (table 6.1) and pertains especially to tumours of less sun-exposed skin areas.

Table 6.1 Increase in skin melanoma incidence (number of cases per 100,000 individuals annually). (Source: Koh91).

	men		women	
	'53-'63	'78-'82	'53-'63	'78-'82
Denmark	1.6	5.9	2.2	8.4
New Zealand (non-Maori)	4.5	15.6	7.7	21.4
Sweden	2.4	7.2	2.8	8.2
USA (Connecticut)[a]	3.0	8.4	3.6	7.7
Israel	2.4	5.8	3.3	7.4
Canada (Alberta)	2.2	4.3	3.3	5.7
Slovenia	1.3	2.4	1.4	2.7
England (Birmingham)	0.9	1.6	1.6	3.3

[a] In 1989 the melanoma incidence in Connecticut was 12 per 100,000.

While the skin melanoma incidence in the caucasian population clearly increased during the past four decades, no increase was observed in the coloured population. The incidence of skin melanomas in the pigmented races is considerably lower. The most common type is acrolentiginous melanoma (see section 6.3.2). Hence, pigmentation of the skin seems to protect against the development of skin melanomas.

It is remarkable that the strong increase in melanoma incidence is accompanied by a much smaller increase in mortality. A partial explanation is that melanomas are now detected at an earlier stage as a result of considerable improvements in diagnostic procedures (McK92a) and public awareness. It is important to emphasise that mortality resulting from melanomas depends strongly on the thickness of the tumour. Despite the improvement in detection, the age-corrected melanoma mortality in the caucasian population in the USA has increased from 1.7 per 100,000 in 1973 to 2.4 per 100,000 in 1988 (Lee92). An observation for which still no sufficient explanation has been offered is the fact that mortality in white males is different for the different birth cohorts. In men born before approximately 1950 a steady increase in mortality has been observed over the observation period, while for men born after 1950 a decrease was found. An explanation might be that it is the thicker melanomas, with a bad prognosis, that are found predominantly in older men (Her91). It could also mean that melanomas that have developed in younger men are of a slightly different character. At present no unequivocal explanation can be given for the change in mortality pattern in the younger age groups.

Incidence and mortality in the Netherlands

Detailed information on melanoma incidence in the Netherlands is not available. Based on data taken from the Pathological-Anatomical National Computerised Archive (Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief; PALGA) the 1986 melanoma incidence in the Netherlands has been estimated at 1270 cases. Recent information from the Netherlands Cancer Registry indicates a melanoma incidence of 1618 in 1989 (626 in men and 992 in women). When melanoma *in situ* are also included, this number increases by 269 cases (96 in men and 173 in women). When all cancers (NMSC excluded) are ranked as to their incidence, melanoma occupies the 6th place in women (12.7 per 100,000 person-years) and the 11th place in men (8.9 per 100,000) (NCR89). In women aged 30 to 44 years melanoma is in third place and in men of the same age group, first. Data obtained for the south-east of the Netherlands indicate a doubling of the incidence between 1975 and 1983 (STG87). Recent consistent data on an increase in incidence are not available. It should be noted, however, that the 1986 PALGA data probably present an incomplete picture, since not all melanomas have been recorded. This means that the incidences for 1986 are probably higher. Therefore it cannot be stated with certainty whether an increase in melanoma incidence really has taken place in recent years. The 1989 data do indicate a relatively high melanoma incidence (approximately 13 per 100,000 person-years). The melanoma incidence in the Netherlands appears to be very high and approximately equals that in Connecticut (USA) in 1989 (see table 6.1).

In the Netherlands more is known about the mortality resulting from melanoma than about the incidence of melanomas, as a result of better records. Nelemans and colleagues analysed melanoma mortality in the Netherlands between 1950 and 1988 on the basis of data from the Dutch Central Bureau of Statistics (CBS) (Nel93b). The annual age-standardised mortality rates have increased four times both in men and women (in men from 0.41 per 100,000 in 1950 to 1.89 per 100,000 in 1988 and in women from 0.39 per 100,000 in 1950 to 1.38 per 100,000 in 1988). In 1950 only 10 men and 10 women died as a result of melanoma, in 1988 this had increased to 164 and 177, respectively. An increase in mortality was demonstrated in these data also for the successive birth cohorts up to 1955. A decrease was found, however, for cohorts born after 1955. The increase in mortality might be due partly to an improvement between 1950 and 1970 in specification of the cause of death. This is consistent with the data from Connecticut that also show an increase in melanoma mortality because of an improvement over the same period in the reporting of the cause of death (Rou88).

Association of epidemiological data and etiological factors

When epidemiological information is used to determine etiological factors, one has to take into account the fact that the developmental pathways of the different types of melanomas are probably different (see section 6.3.2). It is therefore important to stratify the incidence data according to the different clinical types of melanoma.

The first (descriptive) epidemiological data from the USA and Australia showed that melanoma incidence increases the closer to the equator people live (Elw84, Lon87). This was not observed in Europe, and also not for NMSC (see section 6.8.1). Further analysis showed that the melanoma incidence was especially increased in individuals from higher socio-economic classes. A lower incidence and mortality from skin melanomas was observed in people with an outdoor occupation than for people employed indoors. This difference remained even when professions were stratified for socio-economic classes. The distribution of melanomas over the body was not always related to cumulated sunlight exposure. LMM was almost exclusively found on the face and was accompanied by an actinic skin. SSM and NM, on the contrary, were found on the trunk. If sunlight exposure is considered a risk factor, a possible interpretation of these data is that infrequent (excessive) sunlight exposure influences the development of SSM and NM (the intermittent sunlight hypothesis) and that LMM is related to cumulative sunlight exposure (Elw92).

This hypothesis was subsequently tested in epidemiological studies that are now discussed. When interpreting the data it should be realised that there can be an influence of the sunlight sensitivity of the subjects. Individuals who sunburn easily (skin type 1) will, in general, expose themselves for shorter periods to sunlight than individuals who burn less quickly (skin type 3). Also, intermittent exposure to sunlight is hard to assess objectively. While an objective determination of cumulative exposure is, to some extent, feasible on the basis of skin lesions (sunlight-related skin ageing), intermittent exposure can only be determined from data obtained in interviews.

Europe, USA and Canada

In a study that has run in the West of Scotland since 1979, an increase was found for almost all melanoma types. SSM had the greatest increase, while acrolentiginous melanoma did not increase at all. An especially marked increase in SSM was observed on the trunk in males and on the legs in females (McK92a). In 1985 an information campaign was started. Family doctors have a key role both in this campaign and in screening. Since the start of this publicity campaign, there has been an increase in the relative number of thin melanomas (as a result of early diagnosis) and a decrease in

melanoma mortality in women (McK92b)*.

The association between sunlight exposure and melanoma incidence has been studied in Denmark (Øst88), Western Canada (Elw84, Lon87), New Zealand and Australia. Østerlind and Longstreth studied the association between certain (recreational) activities with SSM prevalence. The relative risk of SSM or NM appeared to be slightly increased in individuals that had spent much leisure time outdoors, but there was a decreased relative risk of SSM in outdoor workers. These data could be considered as support for the intermittent sunlight hypothesis, at least for SSM. The studies had the drawback, however, that sunlight exposure could have been better recalled by the melanoma patients interviewed than by the controls (recall bias, see chapter 3) (Wei91b). A comparative study among WW II veterans showed that those who had been stationed in the Pacific theatre had a higher risk of developing melanomas than those who had been in Europe (Bro84).

Australia and New Zealand

Studies performed in Queensland and Western-Australia yielded somewhat different results. The data from Western-Australia indicate an increased risk of SSM associated with some (but not all) recreational activities with sunlight exposure. The study in Queensland did not show a clear association between sunlight exposure on the beach and melanoma incidence (Gre84).

In the Western-Australia study a *decreased* risk of melanomas was observed for outdoor workers. This was not found in Queensland. The interpretation of these data was that the UV level in Western-Australia is already so high that the effects of intermittent exposure during leisure activities are cancelled. These studies did reveal an association between melanoma prevalence on the trunk and abdomen with the wearing of bathing suits.

As for NMSC, a difference in incidence of melanomas was found in Australia between immigrants and the native caucasian population. The relative risk of SSM in persons who migrated to Australia before the age of 15 is considerably greater than for other migrants (Hol84, McM88). The explanation for this is similar to that offered by the committee in the case of NMSC: with childhood exposure more time is available for carcinogenesis and young people have more proliferating cells than do older people. The latter can be observed when the increase in number of *naevi naevocellulares* found especially in young people is considered (Gal90b).

* At this time an epidemiological study on melanoma incidence is in progress in the Netherlands (in the northern part of the province of South Holland). Also, a publicity and educational campaign on melanomas is planned in co-operation with the department of Dermatology of the Royal London Hospital within the framework of 'Europe against cancer'.

Armstrong suggests in a detailed summary of the epidemiological studies that intermittent sunlight exposure (leisure time, sunburn) and, possibly, the cumulative amount of sunlight (actinic skin) are both important factors in the development of skin melanomas. Intermittent sunlight exposure especially seems to increase the risk of SSM on the back (Hol86).

The committee feels that, based on the data from the immigrant studies, the hypothesis that childhood exposure has a major influence on the risk of development of melanoma is better founded than the intermittent exposure hypothesis. The latter is based on epidemiological data that depends strongly on the recall of past exposures of the subjects and thus recall bias may seriously distort the results. This is not the case for the immigrant studies.

Other melanoma risk factors

The epidemiological data mentioned above provide indications of an association between UV exposure and the development of certain melanomas. However, other factors also play an important role in melanoma development (table 6.2).

Naevi and sunlight exposure

The strongly increased risk associated with a high number of naevi (moles) is remarkable. Results of studies into the association between sunlight exposure and the prevalence of atypical naevi are less consistent (for a description of the different types of naevi: see section 6.3.2).

Development of the *naevus naevocellularis* seems to be associated with sunlight exposure. Australian studies showed a higher prevalence of *naevi naevocellulares* in the caucasian population in areas with a high risk of sunlight exposure. Also, a study of 14 to 15-year-old students in New Zealand found an increased number of moles among those regularly exposing themselves to sunlight (Coo92). Also, more naevi were found in students that sunburned faster and had a large number of freckles. The number of naevi found in 15-year-olds in Australia and New Zealand is higher than that in their peers in British Columbia (Canada) (Gal90a). An increase in the number of naevi resulting from an increased sunlight exposure is consistent with the results of the immigrant studies mentioned in section 6.8.2.

In addition to the presence of naevi in general, it is also important to note that individuals with the FAMMM syndrome (see section 6.4 and below), having a genetic predisposition to the development of melanomas, are characterised by the presence of multiple atypical naevi. Such naevi, possibly melanoma precursors (see section 6.3.2), are found sporadically in the general population. The prevalence in the Netherlands is

Table 6.2 Melanoma risk factors (Source: Koh91).

risk factor	relative risk[a]
age > 15 year	88
presence of pigmented lesions, dysplastic naevi + familial melanoma (FAMMM syndrome)	148
presence of dysplastic naevi	7-70[b]
presence of lentigo maligna	10
presence of a relatively large number of benign naevi	2-64[b]
presence of congenital naevus	17-21[b]
white (vs. coloured) race	12
earlier melanoma	5-9
skin melanoma in parent	2-8
immunosuppression	2-8
excessive sunlight exposure	3-5
sensitivity to sunlight	2-3

[a] The relative risk is the proportional increase of the risk of development of melanoma for individuals to whom this factor applies, in comparison with individuals in whom this is not the case; a relative risk of 1 means no increased risk

[b] The variation is based partly in differences in definition

10% (Cri93). An association between sunlight exposure and the development of atypical naevi has so far not been demonstrated. A study among the caucasian population of Curaçao gave negative results in this respect. Such naevi were found more often (prevalence: 15%) in persons younger than 15 years than in older people. A study in the area of Leiden in the Netherlands had the same outcome (Cri93). It is not yet clear whether this increased prevalence is a cohort effect or a natural course of the development of atypical naevi.

Familial malignant melanoma

FAMMM patients generally have a greatly increased risk of melanomas (Tin92). A gene associated with the FAMMM syndrome has recently been located on chromosome 9p (Can92). In several families in the Leiden area (the Netherlands), such a gene was also located on chromosome 9p (Gru93a). In other families, however, a FAMMM-

related gene was located on chromosome 1q. The gene responsible for the FAMMM syndrome will probably be isolated within the next two years. When its structure and function are found, this might lead to a breakthrough in the knowledge on the development of melanoma (Kam94).

Discussion of the epidemiological data on the association between UV light and melanoma

The strong increase in skin melanoma incidence among the caucasian population during the past 30 years is most remarkable. Despite extensive epidemiological research, no direct cause has been found. Most epidemiological studies on melanoma patients assume that sunlight exposure plays a role in the development of these tumours and therefore in the increase in incidence. The increase in SSM is especially marked in skin areas exposed to sunlight only periodically, e.g. the legs and trunk. The increase in incidence has primarily been observed among people from the higher socio-economic classes. These observations have led to the supposition that short intermittent sunlight exposure, and especially sunburn, might play a role in the development of SSM. These data are not consistent, however. Moreover these studies have the disadvantage that they are completely based on memories, that may be 'enhanced' in patients. It is also remarkable that people who stay in sunlight for longer periods of time, e.g. fishermen and farmers, have a slightly lower risk of development of SSM than do intermittently exposed individuals.

Immigrant studies in Australia show that sunlight exposure during childhood, up to approximately 18 years of age, is associated with an increased risk of development of malignant melanoma. On the basis of this, the hypothesis was proposed that childhood exposure is a specially important risk factor. This hypothesis is further supported by the observation that the number of moles increases with sunlight exposure at younger ages and, in turn, a large number of moles increases the risk of melanoma. The possibly increased proliferative activity of pigment cells during childhood makes them more vulnerable to the mutagenic action of UV radiation than naevus cells in older persons (see sections 6.2.2 and 6.8.1).

These epidemiological and cell biological data are in good agreement. Also important in this respect is the observation that patients with a genetic predisposition to the development of melanoma often have atypical naevi. This indicates that atypical naevi might be melanoma precursors. On the other hand it is also possible that a melanoma arises *de novo*, or from a *naevus naevocellularis* without an atypical precursor stage. It is still not clear if and how the observation that atypical naevi are found primarily in young people is related to an association between exposure to UV radiation during childhood and the development of melanoma precursors. Further research, e.g. into the

molecular-genetic background of the FAMMM syndrome, should provide more information about the role of UV radiation in the development of the precursor skin lesions and the progression to CMM.

The data presented above indicate that the association between sunlight exposure and melanoma development is not unequivocal. One can safely assume that melanomas are not induced by any single factor. Other factors besides sunlight exposure may cause, or contribute to, melanoma development. An important factor is the genetic predisposition of an individual. It is possible that the effect of UV radiation on pigment metabolism is also important. Persons with relatively high pheomelanin and low eumelanin, such as those with skin type 1, are at increased risk of melanoma development. In these individuals this is not due to a large number of *naevi naevocellulares*. Some people with skin type 1 have only few naevi. However, other forms of aberrant pigmentation, such as freckles, are quite common and are also well-known risk factors for CMM. The risk of melanoma development is not solely the result of the presence of many naevus cells, but is perhaps more related to pheomelanin. Once pheomelanin is activated by UV radiation it can damage the cell by releasing oxygen radicals. This process can even be reinforced since sunlight exposure can stimulate the formation of pheomelanin. Reactive oxygen species can damage essential molecules in the cell. The exact mechanism of cellular damage is, however, largely unknown.

According to studies by Nelemans an association exits between melanoma incidence and exposure to chlorine-containing water (Nel93a). The hypothesis is that chlorine acts as a co-carcinogen in the development of melanoma due to oxidative stress.

Main conclusions

The main conclusions of the committee are the following:

- The association between sunlight exposure and the development of melanomas is not clear.
- Sunlight exposure during childhood increases the risk of melanoma. Consistent evidence for this is given by results of the immigrant and mole studies.
- The development of certain melanoma types (e.g. SSM) is possibly related to irregular exposure to high-intensity sunlight. The results of studies performed to verify this intermittent exposure hypothesis are possibly influenced by 'recall bias'.
- Genetic factors like the presence of the FAMMM syndrome gene lead to an increased risk of melanoma development. This is also true for the presence of atypical naevi.

Table 6.3 Strength of the indications of a role of UV radiation in carcinogenesis.

		BCC	SCC	MSC
epidemiology	latitude dependence	++	+++	+
	outdoor profession	++	++	—
	exposed skin	+++	+++	+
	sunlight sensitivity	++	++	++
	migration	++	+	+++
	irregular exposure	?	?	±
patients	XP	+++	+++	+++
animals	UV tumours	+	+++	+
	dose-response	?	+++	+
	action spectrum	?	++	±
human tumours	UV-type mutation	+++	+++	+++

+++ sunlight hypothesis strongly supported
++ sunlight hypothesis clearly supported
+ support for sunlight hypothesis
± possibly some support
— no support
? unknown / not investigated

- The effect of UV radiation on pigment metabolism may influence melanoma development. Fair-skinned people (skin type 1) with relatively much pheomelanin and a number of pigment anomalies (e.g. freckles) are at increased risk.

6.8.3 *Summary of indications of a role of UV radiation in carcinogenesis*

In table 6.3 the committee presents an overview of the arguments mentioned in preceding sections that support the hypothesis that UV radiation has a causal effect on the development of NMSC and MSC.

6.9 Quantification of skin cancer risk

Any calculation of the increase in the number of skin tumours due to increased exposure to UV radiation should preferably be based on human data. These data, obtained from epidemiological studies, were discussed in this chapter. They are not sufficient,

however, for proper quantification of the relation between exposure to UV radiation and skin cancer. Such calculations demand information about dose-effect relationships, i.e.:

a precise identification of the effect: which tumours are the result of exposure to UV radiation
b proper definition of 'dose' pertaining to the effect considered and
c the relation between (a) and (b).

The epidemiological data are not unequivocal concerning (a) and the information on (b) and (c) cannot be separated. Experimental data are a prerequisite for a useful quantitative interpretation of the epidemiological data; animal experimental models are especially suitable for a thorough study of UV radiation-induced carcinogenesis.

6.9.1 *Carcinogenic dose*

Epidemiological data only allow the determination of whether the prevalence of a certain type of skin cancer is correlated with sunlight exposure. It is not possible to decide from these data whether a causal relation exists and, if this is the case, which component of sunlight (infrared, visible light of UV radiation) is responsible. Additional data are necessary.

The first evidence for carcinogenicity of UV radiation comes from animal experiments performed in the twenties and thirties (Rof39). Absorption of UV-B by window glass appeared to prevent the development of tumours. It was not possible at that time to give a proper definition of the carcinogenic UV dose.

An adequate animal model using immuno-competent hairless mice (SKH:HR1) has been available since the sixties for the induction by UV radiation of SCC in the skin. Benign precursors of SCC (actinic keratosis and *Morbus Bowen*, see section 6.3.1) have also been observed in experiments with this model. The photobiological group of the former 'Skin and Cancer Hospital' in Philadelphia (USA) and the department of Dermatology of the University of Utrecht (the Netherlands) performed a series of experiments that allowed derivation of the wavelength-dependence of SCC induction by UV radiation (Gru93b). This wavelength-dependence is visualised in the SCUP (Skin Cancer Utrecht Philadelphia) action spectrum (figure 6.4). The maximal effect is found at a wavelength of 293 nm. Using this action spectrum the spectrally weighted exposure can be calculated (see chapter 2) and, from this, the carcinogenic UV dose for the mice.

The same action spectrum can also be used to estimate the carcinogenic dose for humans (Gru93c, Lon91). For this purpose the action spectrum can be corrected for the optical differences between the epidermis of mice and humans*. The original action

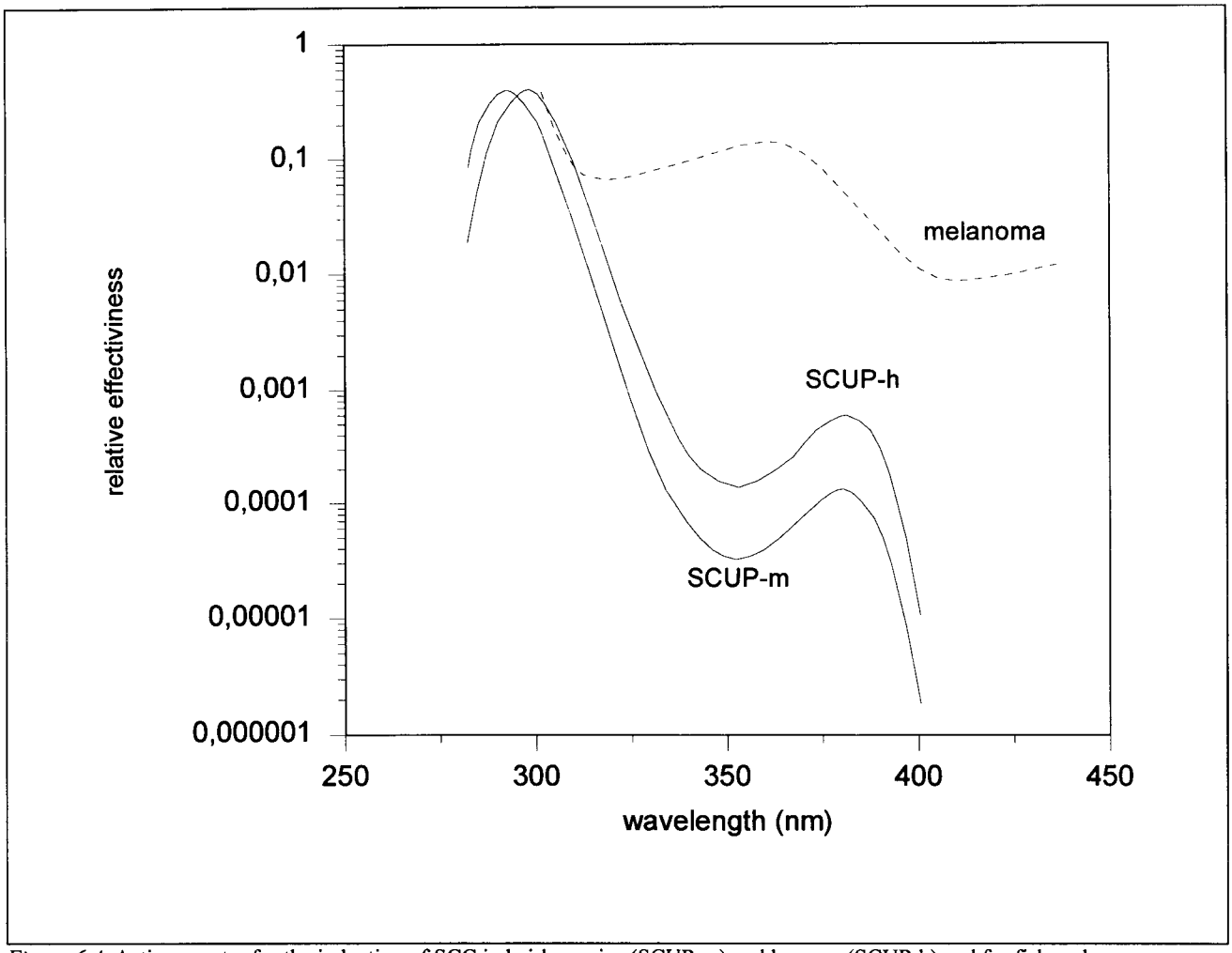

Figure 6.4 Action spectra for the induction of SCC in hairless mice (SCUP-m) and humans (SCUP-h) and for fish melanoma (Source: Gru93b (SCUP) and Set93 (melanoma)).

spectrum is now called SCUP-m (murine), the corrected action spectrum SCUP-h (human) (Gru94). The SCUP-h action spectrum is also drawn in figure 6.4. It represents the average values for skin types 1 and 2. The maximum has shifted to a higher wavelength and the relative effectiveness above 340 nm is increased by a factor of five

* It is likely that UV radiation-induced carcinogenesis takes place primarily in the basal cells of the epidermis. Radiation therefore has to pass through the entire epidermis to reach these cells. Mouse epidermis on the average is thinner than that of humans: in the unirradiated skin of the back, 30 versus 70 μm, respectively. The SCUP action spectrum can be corrected for the corresponding difference in transmission. A complication not taken into consideration here, is the possible thickening of the epidermis by UV radiation, that occurs in both mice and in humans after sufficient UV exposure. There are not enough data on these changes, however, to further refine the correction. For simplicity's sake it is assumed that the real differences in transmission between mice and men under relevant UV irradiations do not strongly deviate from the average for unirradiated epidermis.

compared to the SCUP-m action spectrum. Both spectra resemble the action spectrum for the induction of minimal erythema established by the Commission Internationale de l'Éclairage (CIE), the erythema action spectrum determined 24 h after irradiation (McK87) (see figure 2.2). This is located approximately between the SCUP-m and SCUP-h action spectra. The Health Council report, 'Optical radiation', used this erythema action spectrum to determine the erythema-effective radiation doses (GR93).

The induction of BCC by UV radiation has only been observed very incidentally in animal experiments. Strictly speaking there is therefore no action spectrum for the induction of BCC. However, in view of the parallels with SCC (BCC also occurs on those skin areas most frequently exposed to sunlight, occurs at higher frequencies in outdoor workers and often has UV-related mutations similar to those of SCC in the p53 tumour-suppressor gene) it is plausible that the SCUP-h also can be applied to BCC in humans.

The situation is different, however, for human melanoma. Without a clear picture of the role of UV radiation in the induction of these tumours it is not possible to give a scientifically sound action spectrum. A melanoma action spectrum has recently been published, but it was obtained with irradiation of fish (hybrids of the species *Xyphophorus*) (Set93). This melanoma action spectrum is flatter than the SCUP-m action spectrum. It has a relatively high effectiveness in the UV-A (see figure 6.4). Since melanomas also develop without UV irradiation in about 20% of these fish, it is possible that UV radiation only accelerates the development of latent tumours and thus gives rise to more tumours in a shorter period of time. In another animal model, the opossum *Monodelphis domestica*, melanomas do not develop spontaneously. The tumours induced by UV radiation seem to result from pyrimidine dimers: in the opossum an enzyme, photolyase, is present that repairs pyrimidine dimers after being activated by blue light. Blue light treatment counteracted the development of melanomas (Ley89). The action spectrum for the formation of these dimers (not for the induction of melanomas) has a stronger resemblance to the SCUP-h than to the fish-melanoma action spectrum. It remains to be seen whether these action spectra are applicable to or can be extrapolated to humans.

6.9.2 *Dose-effect relation for NMSC*

Skin tumour incidence, expressed as the number of new cases per year per 100,000 individuals, is proportional to a power function of the annual UV dose (with power $p1$) and a power function of age (with power p) (see equation A), as follows from both epidemiological and experimental studies. The power function of the UV radiation dose implies that the incidence reacts strongly to changes in the UV dose; an increase of the UV dose will result in more tumours at younger ages and in more tumours overall.

Table 6.4 Values for the parameters *p1* en *p* of the cumulative incidence of SCC and BCC (see equations (A) and (C)). The uncertainties indicated are standard deviations. (Source: Gru83 (experimental data), Coe91 (data for the Netherlands), Sco81 (data for the USA)).

Tumour		*p1*	*p*
SCC (∅ 0-1 mm)	mice	4.3 ± 0.5	7.2 ± 0.8
(∅ 4 mm)	mice	4.6 ± 0.5	9.0 ± 0.9
SCC	men (NL)		6.6 ± 0.4
	men (USA)	2.5 ± 0.6	5.6 ± 0.2
	women (NL)		8.9 ± 0.7
	women (USA)	2.5 ± 0.7	5.3 ± 0.3
BCC	men (NL)		5.4 ± 0.1
	men (USA)	1.5 ± 0.4	4.66 ± 0.06
	women (NL)		4.8 ± 0.1
	women (USA)	1.3 ± 0.4	4.74 ± 0.04

When a cohort of individuals of the same age is exposed regularly to UV radiation, skin tumours will develop after some time. The total number of tumours per individual is called the cumulative incidence or yield. This definition deviates from the usual one for fatal diseases since UV-induced tumours are not necessarily fatal. An individual can therefore contract tumours more than once in a lifetime. The dependence of the cumulative tumour incidence *CI* on the daily (mouse) or annual (man) UV irradiation dose *H* and of the experimental period (mouse) or age (man), *a*, can be described as follows:

$$CI = \left(\frac{H}{H_0}\right)^{p1}\left(\frac{a}{a_0}\right)^{p} \qquad \text{(A)}$$

where *p1* and *p* are constants, as are H_0 (in $J \cdot m^{-2}$) and a_0 (in days or years), a reference dose and reference age, respectively. The values $1/H_0$ and $1/a_0$ (rate constants) are proportional to the rates or chances of UV- and time-dependent processes; these values are different for mice and humans. The mouse and human values for the constant *p* for SCC are approximately similar; *p1* seems to be lower for humans. The values of the constants *p1* and *p* for BCC are lower than the corresponding ones for SCC (table 6.4).

The chance P of having (had) a tumour at a certain point in time is

$$P = 1\text{-}e^{(-CI)} \qquad \text{(B)}$$

The age-specific incidence I, the number of new tumours per day or per year per individual, is the first derivative of CI in a:

$$I = \left(\frac{\partial CI}{\partial a}\right)_H = \left(\frac{p}{a_0}\right)\left(\frac{H}{H_0}\right)^{p1}\left(\frac{a}{a_0}\right)^{p-1} \qquad \text{(C)}$$

CI and P are determined in animal experiments. The incidence I for humans (per 100,000 persons instead of per individual) is derived from epidemiological data. Individual annual UV irradiation doses cannot be measured. The best approach is to assume that these doses are proportional to the maximum available annual UV irradiation dose in a horizontal plane in the place of residence. This parameter can be measured or computed.

Equations (A) and (C) show that, if H doubles, CI (or I) increases by a factor 2^{p1} at each age. The CI reached at a certain age a, is already reached at an age $a/2^r$ when H is doubled, where $r = p1/p$. With the values for $p1$ and p from table 6.4 this implies that, when the carcinogenic dose doubles, the tumour induction period for SCC shortens by 39% and that of BCC by 23%.

The calculation of factors $p1$ and p for humans is based on the data of Scotto mentioned in section 6.8.1 (Sco81). In this study the annual UV irradiation doses were measured at several locations, using a Robertson-Berger (RB) meter. The spectral response of these meters approaches the erythema action spectrum, i.e., the meters provide the approximated erythema doses. The approximation is, however, not very accurate. Therefore a correction was applied in the calculation of $p1$ (table 6.4) for the difference between the RB values and the SCUP-h-weighted annual UV dose*.

The values of the age dependence exponent p for SCC in humans are comparable to those for mice. This agreement seems to be better for populations at greater distances from the equator: in the USA the value for p decreases for more southern latitudes (a detailed analysis is given in Gru93c). The $p1$ values for SCC are found to be lower for humans than for mice. This indicates that in humans the induction of SCC depends less on the UV irradiation dose than in the mouse model. This might be attributed to other carcinogenic factors in the human environment that also contribute to the development of SCC.

Since the exponents for time and dose dependence are probably proportional to the number of time- and UV-related steps (e.g. mutations) in carcinogenesis (Gru91), the

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* It has been determined empirically that annual UV irradiation doses that are weighted according to different methods show the following relationship:

$$H_X(L) \propto [H_{RB}(L)]^q \qquad \text{(D)}$$

where L is the latitude, the index 'RB' indicates the RB-weighted UV dose and the index 'X' an X-weighted UV dose. A double-log plot of H_X versus H_{RB} for various latitudes results in a straight line with coefficient q. X = SCUP-h results in $q = 1.10$. When the value of $p1$ for RB-weighting is divided by 1.10, the result is $p1$ for SCUP-h weighting.

lower values for $p1$ and p associated with BCC might indicate that more such steps are necessary in the development of SCC.

6.9.3 *Dose-effect relationship for CMM*

The firmest data from the epidemiological studies are that the risk of development of CMM is increased in individuals that have a sunburn-sensitive skin or a large number of moles or atypical naevi, and for those who have moved from a moderate to a (sub)tropical climate during childhood. The observed increase in CMM incidence in the caucasian population as its proximity to the equator increases is in agreement with this. This information is not sufficient, however, to establish a dose-effect relationship that can be used for a quantitative risk-analysis.

Animal experiments do not provide clear information on a possible mechanism. In hybrid fish of the *Xiphophorus* species a single UV-A irradiation shortly after birth is sufficient for the stimulation of melanoma formation. In opossums, melanomas develop after chronic UV-B irradiation. This information does not allow the determination of a dose-effect relationship that can be extrapolated to the human situation.

It is possible to calculate from the Scotto data that CMM-incidence also shows a slight power dependence of the annual UV irradiation dose as measured by the RB meter. The exponent $p1$ of the UV irradiation dose is in the range of 0.5 to 1.0. In view of the uncertainties concerning the action spectrum for the induction of CMM in humans and the possibility that it is completely different from the erythema and NMSC action spectra, it is not possible to correct the RB values for any CMM induction action spectrum.

6.9.4 *Ozone depletion and amplification factors*

Calculation of the effect of depletion of the ozone layer is a two-step process. Firstly, the effect on the annual UV-irradiation dose is determined and secondly, the magnitude of the increase in skin cancer incidence resulting from the increase in UV dose is calculated.

The 'optical amplification factor' VF_0 gives the percent increase in annual carcinogenic UV irradiation dose for each percent depletion of the ozone layer (O_3, in Dobson Units (see section 2.3.1)):

$$VF_o = -\left(\frac{\Delta H_{SCUP-h}}{H_{SCUP-h}}\right)\left(\frac{\Delta O_3}{O_3}\right)^{-1} \qquad \text{(E)}$$

At 52° north and with 320 DU O_3 VF_o equals 1.2 for SCUP-h-weighted annual UV irradiation doses and 1.4 for the SCUP-m-weighted doses. The carcinogenic UV irradiation dose thus increases by 1.2 - 1.4% per percent O_3 decrease.

The 'biological amplification factor', VF_b, indicates the percent increase of tumour incidence (I) for each percent increase in carcinogenic UV irradiation dose:

$$VF_b = -\left(\frac{\Delta I}{I}\right)\left(\frac{\Delta H_{SCUP-h}}{H_{SCUP-h}}\right)^{-1} \qquad \text{(F)}$$

This can also be written as $VF_b = (\mathrm{d} \log I) / (\mathrm{d} \log H) = p1$. VF_b therefore equals $p1$ in table 6.4. On the basis of this information each percent increase in carcinogenic UV irradiation dose results in an increase of SCC incidence by approximately 2.5% and of that of BCC by approximately 1.5%.

The product of VF_o and VF_b is the amplification factor, VF: the percent increase in tumour incidence for each percent depletion of the ozone layer. On the basis of the above-mentioned data the committee calculated that $VF = 3.1 \pm 0.9$ for SCC and $VF = 1.9 \pm 0.5$ for BCC (in the error ranges for VF those determined for VF_b ($= p1$) given in table 6.4 dominate).

6.9.5 *Increase in the number of cancer cases*

NMSC

Little is known about the incidence and mortality for NMSC. This results from the fact that registration is not obligatory for these tumours and is therefore incomplete. This is especially true for BCC. The numbers given below are thus most likely underestimates, but to an unknown extent. Underestimation of registration is reported from other countries as well (Sco81).

In the most recent publication on cancer incidence in the Netherlands, by the 'Landelijk Overleg Kankercentra', data for skin cancer include only incidence and mortality data for melanomas and 'other skin cancers' (Vis93). BCC is explicitly excluded from the 'other skin cancers'. This category therefore mainly pertains to SCC. The prevalence of invasive SCC in the Netherlands in 1990 was 2459 cases, 1595 in men and 864 in women (Vis93). This too is probably an underestimate of the true number. Based on this information it can be estimated that the incidence of (invasive) SCC in the Netherlands is approximately 160 per million annually. This corresponds well with extrapolation of the SCC incidence data of Scotto (Sco81) to the latitude of the Netherlands: 170 per million annually. A similar extrapolation for BCC results in an incidence of 1000 per million annually.

Extrapolation of the skin cancer incidence data calculated for the south-eastern part of the Netherlands (Coe91) to the entire population of the Netherlands (according to the European Standard population and assuming 15 million inhabitants in total) results in an SCC incidence for the Netherlands of approximately 1700 annually (110 per million annually) and an BCC incidence of 8300 (550 million annually). In view of the numbers mentioned earlier, these probably are underestimates. An explanation might be that only primary tumours were registered in this study, but this cannot fully explain the difference. The assumption is supported by estimates by Neering and Cramer of an annual NMSC incidence in the Netherlands of approximately 1000 per million (Nee88).

On the basis of this information the committee estimates the annual SCC incidence in the Netherlands to be 160 ± 20 per million and the annual BCC incidence, 900 ± 300 per million.

A sustained 10% stratospheric ozone depletion, assuming otherwise unchanged behaviour, could in due course result in 34% more SCC and 20% more BCC. The number of extra SCC cases then is 55 ± 20 per million annually and that of BCC 180 ± 75 per million annually. For a population size of 15 million this amounts to approximately 825 extra cases of SCC and 2700 extra cases of BCC annually.

In figure 6.5 the committee presents the calculated extra NMSC incidence for the CFC production scenarios mentioned in chapter 2. World-wide full compliance with the production limitations has been assumed. The figure shows that the increase in incidence expected as a result of ozone depletion will continue up to the year 2040, even for the most favourable scenario (Copenhagen), to reach approximately 190 extra cases per million people annually in the Netherlands. Comparison with figure 2.6 shows that the peak in the increase in NMSC incidence comes several decades after the peak in the increase in UV-B radiation. This reflects the latency period for the manifestation of skin tumours. After 2040 and with full compliance with the most favourable scenario, the increase in NMSC incidence may gradually decrease.

Melanomas

Because it is possible that the action spectrum for induction of melanomas deviates significantly from that for NMSC, the committee considers it improper and unwise to perform calculations or make predictions on the effect of ozone depletion on the incidence of these skin tumours. Moreover it is also likely that there are vast differences between the different types of melanomas with respect to these effects.

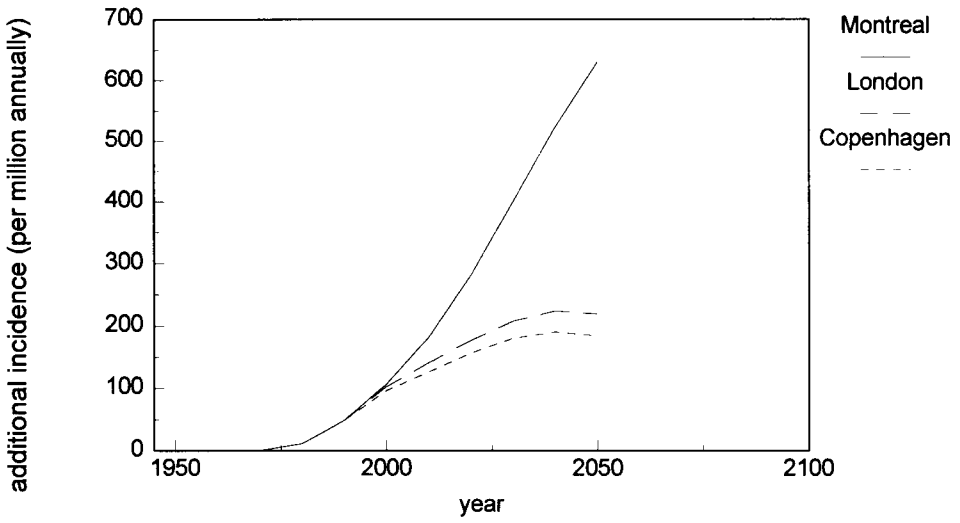

Figure 6.5 Calculation of the increase in NMSC incidence in the Netherlands resulting from ozone layer depletion. In the calculation a middle scenario of the Dutch Central Bureau of Statistics has been assumed for age structure. The calculation is based on the UV-chain model developed by the National Institute of Public Health and Environmental Protection, RIVM (Sla92, RIVM93) using a correction for the applied action spectrum (SCUP-h) and incidences.

6.9.6 *Estimation of mortality*

The risk policy of the Dutch government often uses the additional mortality risk (see section 1.2). With NMSC, the incidence is much higher than the mortality. Data of the 'Landelijk Overleg Kankercentra' show that SCC mortality is approximately 3% of the incidence (Vis93, Win92). Adequate data for BCC are lacking, but based on studies in the USA it can be estimated that mortality for this type of cancer is approximately 0.3%. The average mortality fraction of NMSC depends on the SCC/BCC ratio. For the data shown in figure 6.5 the mortality fraction amounts to approximately 1% of the incidence. It was assumed in the calculations that mortality is a constant fraction of the incidence, independent of age and UV dose.

The additional mortality as a result of depletion of the ozone layer therefore is also approximately 3% of the additional incidence for SCC, 0.3% for BCC, and on the average 1% for NMSC. This means that for a sustained 10% stratospheric ozone depletion, and using the incidence numbers mentioned earlier, there will be an additional number of deaths due to SCC of 1.7 ± 0.6 per million annually and due to BCC of 0.5 ± 0.2 per million annually. For a population size of 15 million this means

approximately 25 extra deaths annually attributable to SCC and 8 extra deaths to BCC. Due to the long latency period for the manifestation of skin tumours, it is likely that this pertains primarily to older people.

It is likely that in reality the additional mortality risk is substantially higher when a contribution of melanomas is included. Several investigators and organisations have used the latitude-dependent melanoma incidence mentioned in section 6.9.3 to estimate the mortality risk (Gru91, Lon87, Sla92, Sla94, UNEP91). Based on these estimations the additional mortality risk per percent UV increase for all skin tumours combined might be twice the 1% mentioned earlier for NMSC alone. However, as a logical consequence of its reluctance in predicting the increase in melanoma incidence, the committee feels it improper to endorse the estimate of additional mortality resulting from melanomas.

Chapter 7

Effects on the eyes

In this chapter the committee presents a short introduction regarding the structure of the eye, followed by an overview of wavelength-dependent absorption of UV radiation in the different parts of the eye. The lesions that may develop or be aggravated under the influence of UV radiation are also dealt with. The main focus is on lenticular lesions, specifically cataract.

Cataract is a lesion of the eye found very frequently, especially in older people. Without treatment cataract will inevitably lead to blindness. Adequate treatment can be provided by surgical removal of the diseased lens and implantation of a new (artificial) lens. As a result of the costs involved and the necessary well-developed ophthalmological care this treatment can only be widely applied in developed countries. In these countries, therefore, senile cataract does not *have* to lead to blindness.

Because of the relatively simple treatment and the absence of several complicating factors, such as the costs of lost years of life, that have to be taken into account in the case of cancer, cataract can very well serve as an example of the burden imposed on the health care budget by a disease in which UV radiation may play a role.

In the Netherlands approximately 31,000 lens extractions were performed in 1989 (Ber91). In 1992 approximately 41,000 operative interventions on the lens of the eye were performed (SIG94). The costs involved, including those of pre- and postoperative care, have risen, to being of the order of 200 million guilders (approximately US$ 120 million). A comparable cost-development is occurring in the USA and this is a cause of great concern (Ste93a). Also, post-operative complications are very frequent after

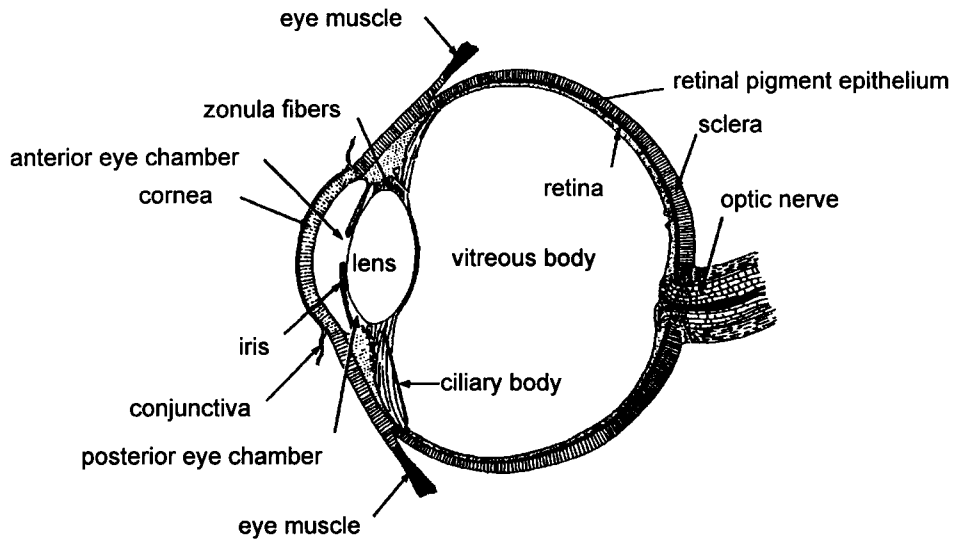

Figure 7.1 Schematic view of the structure of the eye.

cataract surgery. It has been shown that 50-60% of all cornea transplantations are related to preceding cataract surgery (Kap87, Kap92).

7.1 Structure and functioning of the eye

The eye (figure 7.1) consists of several functional and embryologically different elements. The retina is a highly specialised extrusion of the central nervous system, to which it is connected through the optic nerve. Light waves incident on the retina are converted into nerve impulses that are transmitted to the visual cortex of the central nervous system. The other elements of the eye can be considered as specialisations of the epidermis, also having elements of mesodermal origin (supporting cells, glandular tissue, muscle tissue, blood vessels). The cornea, the lens and the vitreous body together are responsible for a clear, bright and focused image of the environment on the retina, assisted by the ciliary muscle and the zonula fibers that are connected to the lens (accommodation) and the iris (diaphragmatic function). The sclera and the internal eye pressure together insure the optimal shape of the eye. Between the sclera and the retina is the choroid, that is responsible for the oxygen supply of the outer layers of the retina. Lens and cornea do not contain blood vessels but receive their nutrients from the liquid of the anterior eye chamber that is produced by the ciliary body.

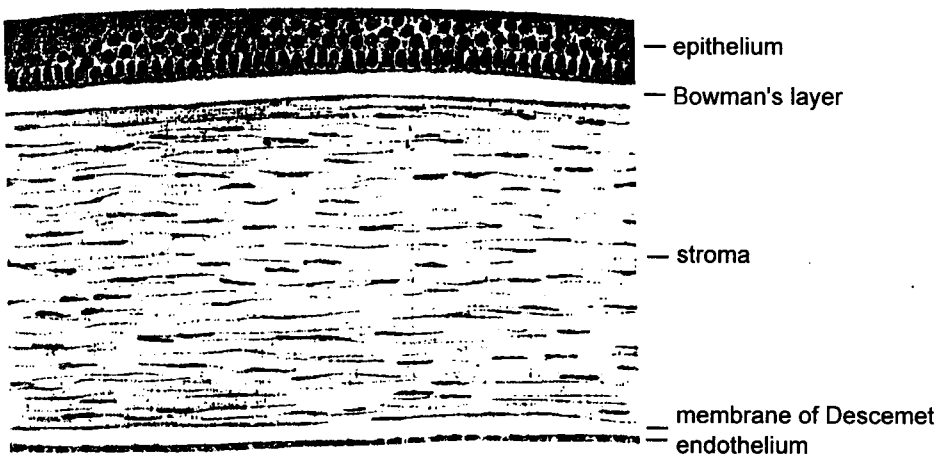

Figure 7.2 Structure of the cornea.

The eye is located in the orbit, bordered on the posterior side by bone and filled with adipose tissue, the lacrimal gland, blood vessels, connective tissue and the peripheral nerves. Also located in the orbit are the six eye muscles that are attached to the eye and are responsible for its movements and a levator muscle for the eyelid. On the anterior side the eye is bordered by two skin folds, the eyelids, the posterior epithelium of which (the conjunctiva) is continuous with the sclera and the cornea. The epithelium of the cornea is in fact a continuation of this conjunctival epithelium.

The elements of the eye that are most sensitive to exposure to UV radiation are the cornea, the iris, the lens, the vitreous body and the retina.

The cornea (figure 7.2) is an approximately 0.6 mm thick transparent tissue covering the frontal side of the eye. On the outside it is covered by a multicellular non-keratinising epithelium that is being renewed constantly throughout life. Ninety percent of the corneal thickness is taken up by the stroma, which is composed of 200-500 parallel lamellae consisting of collagen fibrils. Interspersed between the lamellae are the keratinocytes that produce the collagen. The collagen fibrils are of uniform thickness and equidistant, probably as a result of coupling by specific protein molecules. On the inside, the cornea is covered by a monocellular layer: the corneal endothelium. This endothelium provides nutrients and oxygen to the cornea and regulates its water supply. At birth there are approximately 3500-4000 corneal epithelium cells per mm^2 in humans. This number decreases steadily to 1500-2000 per mm^2 at more advanced

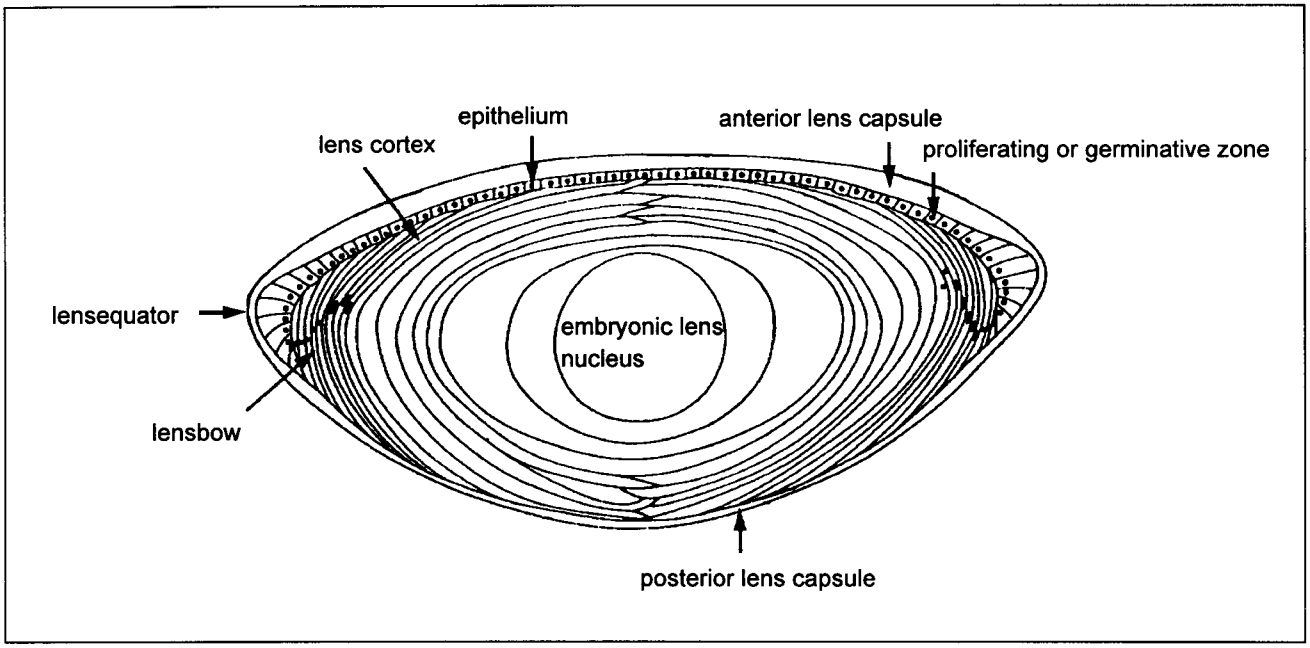

Figure 7.3 Schematic overview of the structure of the lens.

ages (Hop94). As far as is known, no new corneal epithelium cells are formed in humans. Between the epithelium and the stroma and the stroma and the endothelium are two firm collagen layers: Bowman's layer and the membrane of Descemet.

The lens (figure 7.3) is formed at an early embryonic stage as an invagination of the skin. It is firmly encapsulated. On the anterior side underneath this capsule is a monocellular epithelium that continues to proliferate in the periphery throughout life. The newly formed cells grow and elongate anteriorly and posteriorly and form a continuously increasing amount of lens fibers. These fibers lose their nuclei and other cellular organelles and subsequently are nothing more than membrane-surrounded tubes filled with a very high percentage of lens-specific proteins (crystallines). It is assumed that no single lens fiber disappears during life. The thickness of the lens therefore increases continuously.

The stroma of the iris consists of fibroblasts, pigment-containing cells (melanocytes), collagen, nerve fibers and two muscles that are responsible for the change of the size of the pupil. Posteriorly the stroma is bordered by a layer of pigment-containing epithelium that is a continuation of the retinal pigment epithelium.

The vitreous body comprises approximately 80% of the volume of the eyeball and is 99% water. It has a mainly gel-like structure resulting from the presence of hyaluronic acid. Cisterns and cavities bordered by lamellae formed from collagen are located in the gel. Specialised cells, hyalocytes, and, occasionally, macrophages are present. The vitreous body fits the retina closely.

The retina is formed by the neuroretina and the pigment epithelium. The neuroretina is an extremely complex structure formed by a large number of different types of nerve cells, one type of which is connected to the photoreceptors, the rods and cones, which convert light into nerve impulses. Located in the retina are also blood vessels that supply this very active tissue with sufficient oxygen and nutrients. On the posterior side the retina is covered with a layer of pigment epithelial cells. These cells are responsible for the transport of oxygen and nutrients from the choroid to the neuroretina. They also take care of the regeneration of the photopigment and of phagocytosis of the waste products. The sensitivity of the retina to light and colours is not homogeneous. The fovea, or yellow spot, located in the optical axis, has the highest density of photoreceptors, the highest resolution and the best discrimination of colours as a result of the large number of cones. No photoreceptors are present in the retina at the site where the optic nerve exits the eye bulb. This is the blind spot in the field of vision. The periphery of the retina contains predominantly rods. This system of rods is important for vision under low-light conditions. The rods are organised in groups (receptive fields) resulting in a very high sensitivity (minimally two light quanta) but with a low spatial resolution.

As a result of its location in the orbit the eye is partly protected in a natural way against excessive light from the sun or artificial light sources positioned in high places. Squinting, frowning and the pupillary reflex also contribute to the natural protection.

7.1.1 *Absorption of UV radiation*

Light incident upon the eye reaches the interior elements via two pathways. Light passing through the sclera is diffuse and does not contribute to the formation of an image. Light entering the eye through the optical elements subsequently passes the cornea, the anterior chamber fluid, the lens and the vitreous body before reaching the retina. Each of these elements absorbs and scatters some of the light.

The penetration of different UV wavelengths is summarised in figure 7.4 (taken from the 1986 Health Council report; GR86). Essentially, no new data have become available since 1986. Three comments have to be made, however. First: the indicated absorptions in the lens are averages for human lenses older than 25 years. Especially in younger lenses, below 10-20 years of age, absorption in the lens is considerably less in the entire UV range. This results in a greater part of the UV radiation reaching the retina in young people (Ler89, Wea88). Second: the absorptions shown for the cornea and lens mean that, in adults, only an extremely small percentage of the UV radiation reaches the retina. This has far-reaching consequences for the estimation of the risks of exposure of this part of the eye which is most important for vision. Finally: with intense sunlight the eye will activate the natural protection mechanisms: squinting,

frowning and narrowing of the pupil. This will considerably reduce the UV burden of the periphery of the cornea and of the lens. The peripheral part of the lens, the proliferating or germinative zone (figure 7.3) with the largest population of dividing cells, will be specially protected against the direct effects of UV radiation.

7.1.2 *Eye lesions*

The two lesions that are most threatening for vision for which reliable scientific data are readily available and that are possibly related to damage from UV radiation have names that link them to ageing: senile cataract and senile macular degeneration or degeneration of the central part of the retina. This reflects current belief that these lesions are the result of a congenital, endogenous ageing process, or that they are connected with a long-term action of noxious environmental factors, e.g. UV radiation. Whatever the exact cause, ageing results in an increased risk of these lesions appearing. A large and highly varied number of factors are considered as risk factors for the above-mentioned lesions: age, cigarette smoking, high blood cholesterol levels, beer drinking, work at a military base, drinking of milk, colour of the iris, exposure to sunlight and a number of others (Har89a, Mül91, Sch92b). The relative risks attached to these factors differ considerably among the various studies. Current belief is that all these factors, together with hereditary characteristics, have some influence.

7.1.3 *Ageing and vision*

Since lesions of the lens are more prevalent than retinal lesions, it is commonly thought that reduction of vision during ageing is primarily caused by alterations in the lens and other media. The effects of UV radiation on these parts of the eye are dealt with in the next sections. This view is challenged by Weale (Wea89), who considers alterations in the pigment epithelium of the retina as the most important factor for the reduction of vision. There are no indications, however, that UV radiation plays an important role in the development of senile macular degeneration. The UV burden of the retina is only a very small fraction of that of the cornea and the lens (see figure 7.4).

7.1.4 *Limitation of the range of wavelengths and of the eye lesions*

This report is limited to damage caused by UV radiation, i.e. invisible electromagnetic radiation with wavelengths up to 400 nm. It is obvious, however, that radiation-induced biological damage to the retina, lens and cornea does not stop at this arbitrary borderline. Strong indications exist that changes in the retina occur due to blue light (Put93). The report 'Optical radiation' that was recently published by the Health

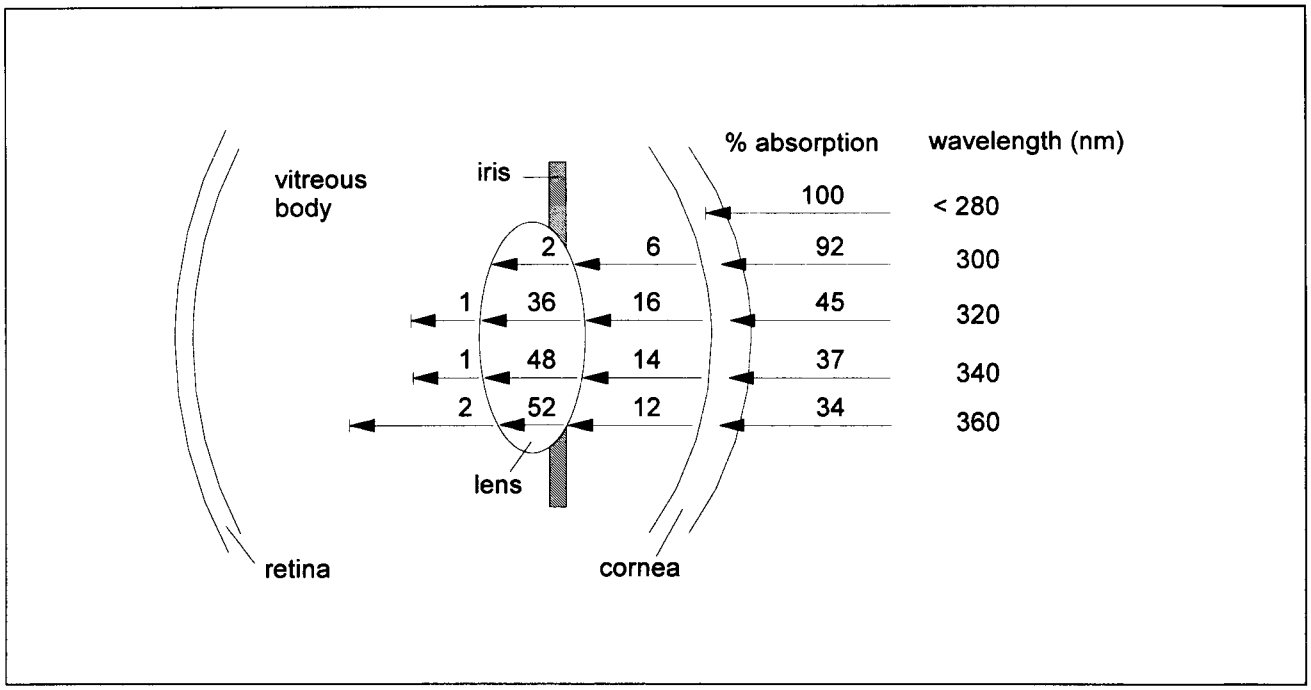

Figure 7.4 Wavelength-dependent penetration of radiation in the eye. The numbers indicate the percentage absorption of incident radiation at the indicated wavelengths by different structures of the eye. (Source: GR86).

Council (GR93) deals with the effects of visible light and infrared and ultraviolet radiation (wavelengths between 100 nm and 1 mm).

In the following discussion on the effects of UV radiation the committee deliberately does not consider traumatic exposures. Development of this type of lesions, called 'snow blindness' or 'welders' eyes', depending on the circumstances of its development, is highly determined by behaviour. It develops after a short very intense UV exposure that affects the corneal epithelium. Snow blindness needs an exposure of several hours, but several seconds may be enough for welders' eyes. This results in very painful damage to the cornea which disappears spontaneously after one or several days. The lesion can be prevented by wearing adequate, UV-blocking, sunglasses during winter sport activities and, for welders, by wearing adequate protective devices.

There are indications that exposure to UV radiation leads to activation of a dormant infection with *Herpes simplex* in the cornea or in the lips. There are no indications that exposure to UV radiation causes bacterial or viral lesions of the eye, nor that systemic alterations that lead to severe lesions of the eye, e.g. in diabetes, are in any way related to UV exposure. These lesions also are not considered here by the committee.

7.2 Lenticular lesions

7.2.1 *Introduction*

During ageing the lens goes through a number of changes that diminish its functioning.

Yellowing

Yellowing of the nucleus of the lens develops even before the third decade of life. This is caused by the light (including UV)-induced formation of chromophores and fluorophores. This yellowing increases with progressing age and the nucleus of the lens may become brown to dark-brown. At the same time, proteins in the nucleus are altered by the formation of disulphide bridges between the proteins and between proteins and glutathione. This oxidative process finally leads to the formation of large, water-insoluble macromolecules. These two processes are termed nuclear cataract. Initially the yellowing has a beneficial effect for the eye, especially for the retina, since UV-A and UV-B are absorbed by the chromophores and cannot reach the retina. The high-molecular weight proteins induce an increase in the scattering of light which has a negative effect on image quality. The excess of scattering elements generally does not increase strongly until the sixth decade.

Opacification of the lens cortex

In contrast to the nucleus, changes in the lens cortex only become manifest in a later phase of life. At the end of the fourth decade milky opacifications develop in the equator or periphery of the anterior lens cortex that completely block the passing of light. These are local lesions of peripheral parts of groups of lens fibers (Vre89, Vre90). It is not yet completely clear what causes them. Changes in the concentration of calcium ions, oxidation of membrane lipids and of the lenticular proteins as well as compression of the firmer lens nucleus during accommodation are considered important causative factors. These alterations are indicated as cortical cataract. Since the local opacifications develop in the periphery of the lens, outside the pupillary opening, at first glance it does not seem very likely that cortical cataract develops as a direct consequence of UV irradiation. It is possible, however, that as a result of the refraction by the cornea, anterior chamber fluid and lens, oblique (UV) light is concentrated on a section of the lens that is shielded from direct light (Cor90). Although it starts to develop in the fourth decade, cortical cataract only becomes manifest in the pupillary opening in the seventh. A disabling cataract can be formed within several months to

years. The reason for this discrepancy between the onset and growth of these early opacities is the encapsulation of the early lens fiber alterations (Vre91). It is still not known why this protective mechanism collapses at later times.

Posterior subcapsular cataract

A serious, but fortunately less frequent, age-associated lesion of the lens is posterior subcapsular cataract, that is located in the cortex on the posterior side of the lens. It consists of an opaque aggregate of abnormally grown cells that fully blocks the normal light transmission of the lens. A possible cause is a change in the DNA of a group of proliferating epithelial cells that leads to abnormally growing fibers with abnormal proteins and a disturbed water balance. This lesion also has its origin outside the pupillary opening. This type of cataract is frequently encountered in diabetics.

Loss of plasticity

Finally alterations occur in the plasticity of the lens, resulting in marked changes in its accommodation width during ageing (from 18 to 0.25 dioptre). This occurs mainly in individuals over 50 and leads to loss of sharp vision at short distances (presbyopia).

7.2.2 Prevalence

Cataract is the most frequent eye disorder. Prevalences of approximately 60% for lenticular lesions and 16% for disabling senile cataract are indicated for the population aged over 50 years. At higher ages (75-85 years) these values may increase up to approximately 90% for lenticular lesions and 46% for cataract. The prevalence is generally higher in women than in men and higher in negroids than in caucasians. Grossly, these prevalence data do not differ much in the developed countries (Kah77, Sas87, Vre89). In India and many African countries there is a shift of the higher prevalences to lower ages (Har91, You91).

Cortical cataract and other lesions of the cortex are most frequent. Up to the age of 60 almost all lenticular lesions are located in the cortex. In people aged over 60 years the prevalence of nuclear cataract increases slightly. The prevalence of pure cortical cataract and a combination of cortical and nuclear cataract is similar around the age of 80 years (Sas87). In the younger age groups posterior subcapsular cataract is the most important clinical reason for cataract surgery. This type of cataract results in such a decrease in the clarity of the lens that surgical intervention is needed immediately.

7.2.3 *Ultraviolet light as a cause of cataract*

Experimental studies

It has been proved that UV radiation has a direct effect on the lens and is capable of inducing lesions in the cortex and nucleus of the lens (Har91, You91). In *in vitro* studies with both intact lenses and isolated lens proteins it has been shown that UV irradiation damages the integrity of proteins and of membrane lipids and leads to the formation of high-molecular protein complexes with strong light-scattering capacities and to disturbance and opacification of the lens fibers. UV irradiation also results in the formation of chromophores and fluorophores. This is most likely mediated by the formation of oxygen radicals. There are also convincing arguments for the assumption that, in experimental animals *in vivo,* ultraviolet light leads to alterations in the lenticular epithelium that may end up as cataract. UV-B is most effective in this respect.

The argument against the predictive value of animal experiments for the effects on the human eye has been raised that many of these experiments are performed on rats, mice and rabbits (Har91). These are nocturnal and twilight animals and it seems likely that they have almost no intrinsic protection against excessive light exposure. Studies are mostly done with young animals that cannot yet have formed substantial amounts of absorbing chromophores. In the only study on daytime animals (adult) squirrels were exposed daily to UV radiation for more than a year. The results, however, did not differ from those obtained with nocturnal or twilight animals (Zig91). Moreover *in vitro* studies with intact lenses do not point to great differences in the effects of UV radiation on human or animal lenses.

Epidemiological studies

The question of whether UV radiation really is a causal factor in the development of human cataract can only be answered on the basis of results of epidemiological studies. In chapter 3 the committee listed a number of criteria that are of importance in the assessment of such studies: consistency, strength of the relation, temporality, dose-response relation and biological plausibility. The last condition seems to be met, based on the information presented earlier. Compliance with the other criteria will have to be demonstrated using the results from the epidemiological studies.

A significant number of these studies have been published in which a correlation is somehow made between sunlight exposure and the prevalence of cataract. These studies can be divided grossly into two groups. In the first group, exposure was estimated based on environmental factors such as geographical location and (natural) protection

from direct sunlight. In many of these studies behavior-determined differences in UV-exposure, e.g. indoor or outdoor employment, wearing of hats and sunglasses, taking a siesta, and differences in ethnic composition, feeding habits and health status were not taken into account. In the second group, attempts were made to determine individual exposure as accurately as possible, on the basis of data on exposure to and protection from sunlight, in combination with information on environmental factors. Studies from this last group allow the establishment of a dose-response relationship. It is often difficult to determine consistency between the different studies, especially since the definition of cataract is variable, certainly so in the older studies. Most cataracts are of the mixed type, but they are sometimes characterised as cortical, nuclear or posterior subcapsular cataract, based on dominance of one type.

The study that best fulfils the above mentioned criteria and that is generally considered to be the most relevant is the one performed on the Maryland Watermen (Tay88, Tay89a). This is a well-defined group of men varying in age from approximately 30 to 90 years that had worked in the ten years preceding the study as fisherman on Chesapeake Bay, in Maryland, USA. The study was such that a number of conditions could be determined reasonably well and with this information the results could be corrected for a number of confounding factors. Individual exposure could be determined with reasonable accuracy and the variation was great enough to allow determination of a dose-dependence. This study showed a positive relation between opacifications in the anterior cortex of the lens and exposure to sunlight. This is considered an indication that UV radiation can play a role in the development of cortical cataract. A similar relation was not found for alterations in the nucleus of the lens and for posterior subcapsular cataract. A case-control study among posterior subcapsular cataract patients in the same region in Maryland did show a weakly positive relation with sunlight exposure (Boc89).

Other studies do not yield strong indications for a relation between sunlight exposure and any form of cataract, some as a result of limitations in study size, others as a result of suboptimal or otherwise faulty study design. The results can be considered, however, as supporting the results of the Maryland Watermen study (a review is given in WHO93).

Based on the experimental and epidemiological data the conclusion seems justified that UV radiation plays a role in the development of senile cataract. Harding has mentioned several arguments that challenge the relation between UV exposure and cortical cataract (Har91). The peripheral lens cortex is hardly exposed to light and the central and peripheral cortex are also the youngest parts of the lens. If UV exposure indeed is an important factor, it must be taken into account along with exposure to light. UV exposure is related to, among others, the natural variation in thickness and concentration of the ozone layer. The facts that the prevalence of cortical cataract is much higher

than that of nuclear cataract, while cortical cataract develops in the not or hardly exposed periphery of the lens (see section 7.2.1) and that UV exposure of the nucleus is much greater and for longer periods must also be taken into consideration. Harding concludes that the higher prevalence of senile cataract in tropical regions, that has been unequivocally shown in several epidemiological studies, is related to the often poor food and hygienic conditions in these areas (Har91). These factors and the associated dehydration and malnutrition resulting from periods of severe diarrhoea are suggested as being much more important causal factors than exposure to UV radiation. Harding even believes that UV is not a causal factor at all.

Based on this review of the literature the committee feels that exposure to UV radiation probably plays a role in the development of cortical cataract, possibly in that of posterior subcapsular cataract, and that no association has been found with nuclear cataract. The committee also concludes that it is not possible, based on available evidence, to determine the relative importance of UV radiation among other possible causal factors, such as diabetes and malnutrition, for the development of cataract. The committee does want to indicate at this point the size of the relative risk (RR) of cortical lesions as found in the Maryland Watermen study in relation to the RRs found for other factors. A relative risk of 3.3 was found for the group exposed to the highest level of UV radiation (Tay88, Tay89a). In other studies a RR of 5.0 was found for the association between cataract and diabetes, an RR of 3.1 for high blood pressure, an RR of 6.7 for coronary artery disorders and an RR of 3.4 for other heart disorders. Chronic bronchitis, hyperlipemia and the use of diuretics are associated with RRs of 4.3, 3.4 and 11.6, respectively. The RRs for smoking and drinking are 2.0 and 2.1, respectively, and that for severe diarrhoea is 2.3.

The relative importance of UV radiation depends strongly on various external factors, e.g. behaviour. Considering the effect of ozone depletion it is important to realise that this will primarily result in an increase in UV-B radiation. Therefore it cannot be excluded that an increase in UV exposure resulting from ozone layer depletion will have an effect on the prevalence of senile cataract, but this effect cannot be quantified.

7.3 Abnormalities and disorders of the cornea, conjunctiva and sclera

There are almost no data available for the prevalence of abnormalities and disorders of the cornea, conjunctiva and sclera in relation to chronic UV irradiation. It is clear, however, that their prevalence is considerably less than that of senile cataract and senile macular degeneration. Corneal transplants are performed on approximately 900 patients annually in the Netherlands. This is only a fraction of the approximately 41,000 cases of cataract surgery. Moreover the cause of corneal opacification is often

the direct or indirect consequence of a preceding ophthalmological intervention, primarily cataract surgery, as noted in section 7.2.2, or of trauma or genetic predisposition.

Two disorders of the limbus, the white continuation of the cornea through which almost no light passes, are connected to exposure to UV radiation. Both sequelae, pterygium and pingueculum, are degenerative yellowish lesions of the conjunctiva, mostly proximal to the nose. Pingueculum is mainly caused by uncontrolled growth of collagen in the stroma of the conjunctiva, while the epithelium is mostly normal. Pterygium is a wing-shaped flap of the conjunctiva infiltrating the superficial cornea. Pterygium is often preceded and accompanied by pingueculum. Exposure to UV radiation is considered to be the most important causal factor. The prevalence of both lesions increases in populations living closer to the equator. The epidemiological study on the Maryland Watermen provided evidence for the likelihood that this relation was present (Tay89b). Wind and salt, in addition to UV radiation, could also have played a role in the development of pterygium and pingueculum in these people (WHO93). The same holds for tropical areas where, in addition to UV radiation, drought and sand in desert areas can also be possible causes (Tay81).

As mentioned in section 7.1 the density of the endothelial cells of the cornea decreases considerably during ageing. This decrease is not linear, but is greater between 0 and 40 years than between 40 and 80 years. The absorption of UV-B by the stroma increases significantly with age (Ler84). It might be concluded on the basis of this that the strong initial decrease in endothelial cell density is the result of excessive UV exposure, and that it is weakened by the increasing absorption of UV-B by the stroma during ageing. A recent study from Japan, however, argues against this hypothesis (Mat85). It shows that the decrease in endothelial cell density is not only considerably less in the Japanese population but is also does not show the typical S-shape.

In summary, there are few indications that exposure to UV radiation is an important causal factor in the development of chronic lesions of the cornea, conjunctiva and sclera (WHO93). The reason these abnormalities occur so much less frequently than lesions of the lens and senile cataract probably lies in the specific histological and biochemical characteristics of the epithelium, stroma and endothelium, which may intrinsically protect against UV damage.

7.4 Abnormalities in the iris and the vitreous body

There are no data available on the relation between UV exposure and lesions of the iris and the vitreous body. It is possible that iris tumours are related to exposure to UV radiation, but no conclusions can be drawn as these lesions are extremely rare.

7.5 Abnormalities and lesions of the retina

Senile macular degeneration is the most prevalent cause of blindness world-wide and is so far not treatable. The prevalence of this lesion is comparable to that of senile cataract, only the age at which there is a strong increase in the prevalence is significantly higher in the case of macular degeneration: between the seventh and eighth decades the prevalence increases from 4% to 12% and in the ninth up to 30% (Sch93b). In some studies a difference is found between men and women. The prevalence is greater in caucasians than in negroids. As already indicated, the risk factors for senile macular degeneration are just as varied as they are for senile cataract, and sunlight exposure is one of these factors. There are not many reasons, however, to assume that UV plays an important role. Figure 7.4 shows that, as a result of absorption in the cornea, lens and vitreous body, virtually no UV radiation reaches the retina in adults. It does so up to the age of 20, but this age group shows no retinal lesions that have a non-traumatic origin. It can be argued that the basis for this degeneration is laid during this period, but that does not explain why it does not become manifest until an advanced age. Abnormalities in the pigment epithelium, the limiting Bruch's membrane and the blood vessels in the sclera are considered the most important causal factors. It is outside the scope of this report to deal with these in detail.

7.6 Conclusions

Available information as to possible involvement of UV radiation in the development of lesions of the eye indicates a causal relation in some cases:

- UV radiation is probably involved in the development of cataract. This applies particularly to cortical cataract and to a lesser extent to posterior subcapsular cataract.
- The development of two lesions of the cornea and conjunctiva, pterygium and pingueculum, is also probably related to exposure to UV radiation.
- The influence of UV radiation in the development of senile macular degeneration is probably minimal, because the cornea, lens and vitreous body almost completely absorb this component of the light.

- As far as chronic lesions of the iris and the vitreous body are concerned, there are almost no indications of a role of UV radiation.

The committee has already argued that it does not feel it is scientifically justifiable to quantify the effects of UV radiation on (parts of) the eye, if such effects are present under normal circumstances. This means that it is also not possible to determine the effect of an increase in UV levels in the Netherlands as a result of ozone depletion, on the incidence of lesions of the eye.

Chapter 8

Effects on aquatic ecosystems

8.1 Introduction

In deep water, almost the entire algal component of the ecosystem consists of micro-phytoplankton. In shallow waters, macro-algae are more important than micro-algae; higher plants that have been adapted to life in water are also found here: reeds, for example, and other water plants and, in the sea, sea-grasses and mangroves. All these primary producers use photosynthesis to produce organic compounds that are taken up by other organisms for their growth. In the aquatic environment viruses, bacteria and protozoa are important components of the food web, but larger organisms higher up in the food chain are more prominent: water fleas, copepods, fish, birds and mammals (otters, seals and dolphins in fresh and salt water, whales in the oceans).

Since light is necessary for photosynthesis, and since light only penetrates in the upper water layer, most of the aquatic ecosystem on earth is located at or closely beneath the surface of lakes, seas and oceans. This layer, called the euphotic zone, therefore has a key position in geochemical element cycles, the cycles of substances that are governed by biological processes. Two of these cycles, the carbon and sulphur cycle, are especially important because of their influence on global climate. The carbon cycle is related to the issue of global atmospheric carbon dioxide increase. The result of such an increase might be an increase in the mean global temperature. The sulphur cycle is important because the upper layer of the ocean is a source of dimethyl sulphide (DMS) and carbonyl sulphide (COS), volatile sulphur compounds that stimulate cloud formation. The micro-organisms of the open ocean are very important in both cycles (figure

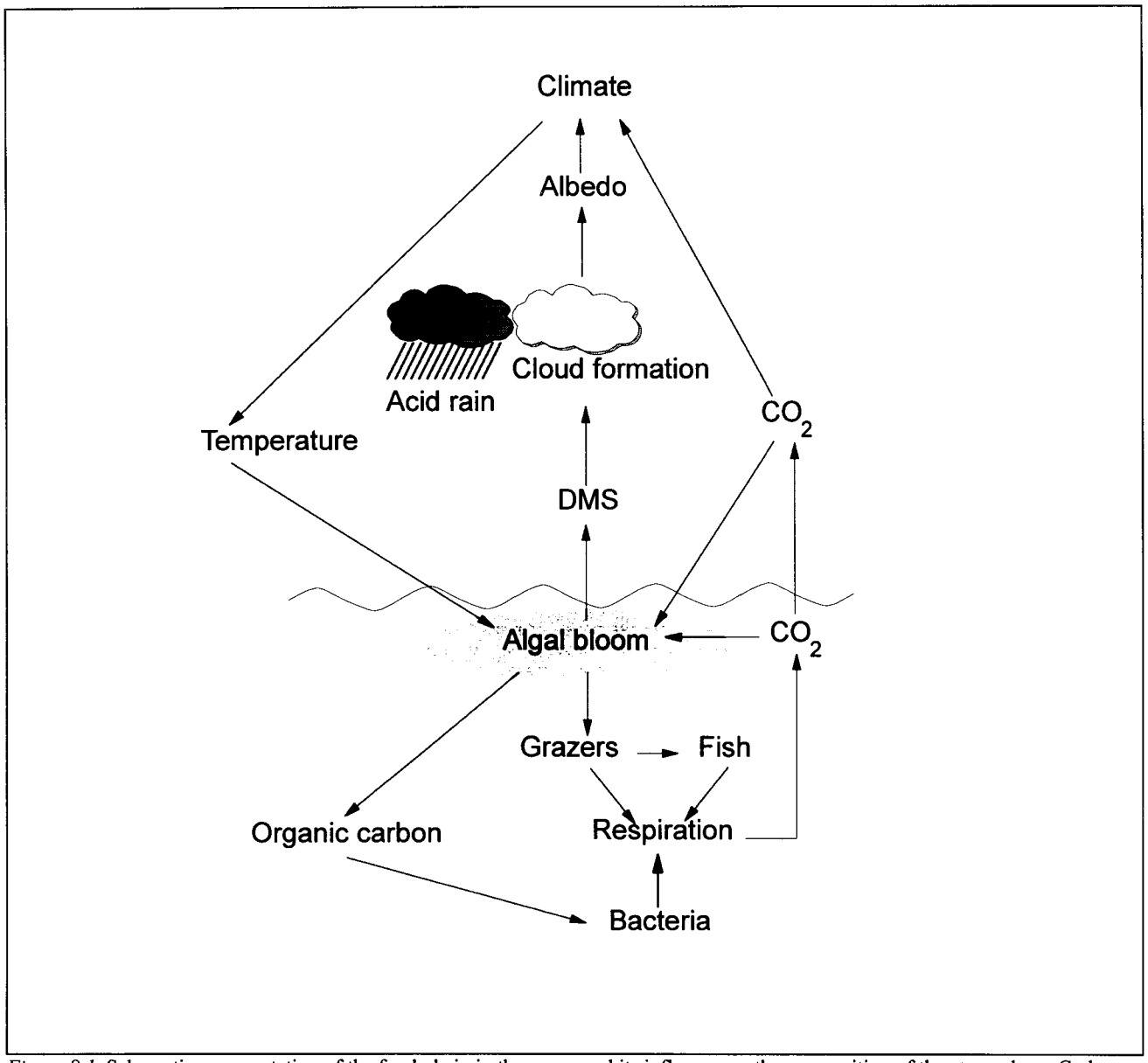

Figure 8.1 Schematic representation of the food-chain in the ocean and its influence on the composition of the atmosphere. Carbon dioxide (CO_2) is the most important greenhouse gas. Exchange of CO_2 between sea water and air and between the different components of the food-chain occurs easily. Dimethyl sulfide (DMS) is a volatile sulfur compound released in areas with algal bloom. DMS influences cloud formation and increases the acidity of rain. The influence of UV-B on algae therefore has direct consequences for world climate as a result of the central position of these organisms in the carbon and sulfur cycles .

8.1). Disturbance of the aquatic ecosystem can therefore result in climate changes.

Processes that are typically confined to the upper layer of oceans, seas and lakes also have consequences for the production of proteins for human consumption: not only almost all fisheries, but mariculture also, take place near the surface.

In this chapter the committee gives an overview of the various aspects of exposure of aquatic ecosystems to UV radiation. The processes described have not yet been investigated to the extent that quantification is possible. The committee therefore cannot estimate the risks an increase in UV radiation carry for aquatic ecosystems. It does indicate what research should be performed to allow such estimates to be made in the future.

The committee feels that the methods used to assess risks for ecosystems as described in the publication, 'Dealing with risks' (TK89), cannot be applied to the effects of UV radiation. The principal reason is that UV radiation, as described in this and the next chapter, influences not only individuals and species but also several abiotic elements of ecosystems and element cycles.

8.2 Penetration of UV-B radiation in water

It is often assumed that ultraviolet light barely penetrates water. However, it was established as early as in the sixties, that short-wave UV-B can be measured up to a depth of several meters, even in coastal waters such as those of the North Sea (Jer74, Jer76). In clear ocean water UV-B penetrates several tens of meters (Bak82). The seas around the Antarctic are among the clearest in the world (Gie87) and it is especially here that the strongest increase in UV radiation has been observed in the past 15 years, in early austral spring when the 'ozone hole' develops.

The submarine spectral composition of visible and ultraviolet light is determined by absorption and scattering, both by water molecules, by dissolved substances and by suspended particles. The most important UV, especially UV-B, radiation-absorbing dissolved substances are the humic acids, that originate from plant material. In the literature these are termed 'yellow substance', 'Gelbstoff' or 'Gilvin'. Algae cells floating in the water absorb and scatter visible and UV light. The pigments of the algae have their absorption maxima primarily in the UV-A and visible range of the spectrum. The amount of chlorophyll is a relatively unimportant factor in the attenuation of UV-B, since other pigments (carotenoids) absorb more in this wavelength range. Material originating from dead organisms also plays a role in the extinction of light. These detritus particles absorb preferentially UV-B radiation, as do the water-dissolved humic acids just mentioned.

During the winter period, the layer of ice and snow covering rivers, lakes and even oceans can also attenuate light in aquatic environments. The spectral composition of

the passing light then is determined by the thickness and structure of this layer. The depth at which UV-B can still be measured further depends on the angle of the sunlight incident on the water surface. This implies that, everywhere on earth, there is not only a clear daily cycle in UV irradiation but also a strong gradient from the tropics to polar regions. In the latter regions there also is a great difference in length of the day between seasons. In summer the biologically effective dose in polar regions is therefore approximately equal to that in tropical areas.

8.3 Effects of UV radiation on geochemical processes

In this section the committee gives an overview of the geochemical processes induced in aquatic ecosystems by UV radiation. The information was mostly summarised from the literature on marine research. Photochemical studies of freshwater ecosystems are scarce. However, since it can be expected that the relevant processes are almost not or not influenced by the salt content, the information available can also be applied to lakes and rivers.

The main role in the induction of changes in inorganic and organic compounds in the aquatic environment is played by the formation of radicals through UV radiation. Direct photochemical reactions also may lead to alterations in the chemical environment of aquatic organisms. This is especially important for the availability of trace elements that are necessary for the growth of algae. The light-directed speciation of metals (the formation of different physico-chemical forms of the same metal), especially that of iron, deserves special attention, because there are strong indications that the availability of iron is a limiting factor in the growth of algae in large parts of the ocean (Mar88b, Pal91). In the USA, speciation of iron is presently a focus of the research directed towards the role of UV radiation in aquatic ecosystems.

8.3.1 Photochemical production of free radicals

The absorption of sunlight by aquatic organic and inorganic compounds causes the direct and indirect formation of various free radicals: hydroxyl radicals (OH·), bromide radicals (Br_2^-), carbonate radicals (CO_3^-), superoxide (O_2^-) and organic radicals. The production rates of the first three are probably highly correlated.

Hydroxyl radicals can react with the surface of living cells and with components of the plasma membrane of both algae and other marine organisms (Pal91). It has been demonstrated in different ocean waters that OH· is formed under the influence of sunlight at rates of 10 - 100 $nM \cdot l^{-1} \cdot h^{-1}$ (Mop90). The production of OH· is strongly determined by the amount of UV-B reaching the surface of the water. OH· reacts especially with bromide in sea water. The bromide radical thus formed is less reactive than OH·.

It has therefore been suggested that the bromide radical can accumulate to considerable 'steady state' concentrations in the sea. It is likely that marine algae are not significantly influenced by extracellular OH· (Pal91).

The influence of the superoxide anion, O_2^-, and of its reaction product, hydrogen peroxide (H_2O_2), is probably much greater than that of OH·. The O_2^- steady state concentration, 1 - 10 nM, is higher than that of other free radicals. Superoxide reacts rapidly with trace elements bound to the cellular surface, such as copper that catalyses the transformation of superoxide into hydrogen peroxide. The formation of hydrogen peroxide in surface waters is correlated to light intensity (figure 8.2), as could be expected from the light-dependent formation of the starting product, O_2^-. At high (mM) concentrations, hydrogen peroxide is toxic to algae. Although the concentration of H_2O_2 in sea water is approximately 0.1 mM, it can reach 34 mM in rainwater.

An important source of free radicals is dissolved organic carbon (DOC). The concentration of the free radicals formed from DOC is not easy to determine, since it is difficult to determine the intermediate products, as a result of the complicated and partly unresolved structure of DOC. This is particularly true for organic radicals. An increase in the amount of UV radiation will result in the formation of more free radicals and photochemical intermediates per quantum, since the absorption by DOC is strongest at the shortest wavelengths. A 30-nm shift of the present incident sunlight spectrum to shorter wavelengths may double the formation of O_2^- (Haa90). The real shift expected from ozone depletion is estimated at only 1 - 2 nm, however.

8.3.2 *Production of gases that can influence the greenhouse effect*

Carbonyl sulphide (COS)

Carbonyl sulphide is a highly persistent volatile sulphur compound that induces cloud formation in the stratosphere, and therefore influences the amount of UV radiation reaching the surface of the earth. COS is formed in the upper layer of ocean waters with a high content of organic matter, probably during the oxidation of sulfur-containing organic substances by hydroxyl radicals (OH·). In open oceans the water is saturated with COS and the COS content varies with the light-dark cycle. The total flux of COS to the atmosphere, expressed as sulphur, is 0.35 million tons annually. This is only very little in the context of the total flux of sulphur from sea to atmosphere, which is 20 - 58 million tons annually but, because of the persistence of COS, it cannot be neglected. The COS flux to the atmosphere on land is partly mitigated by forests.

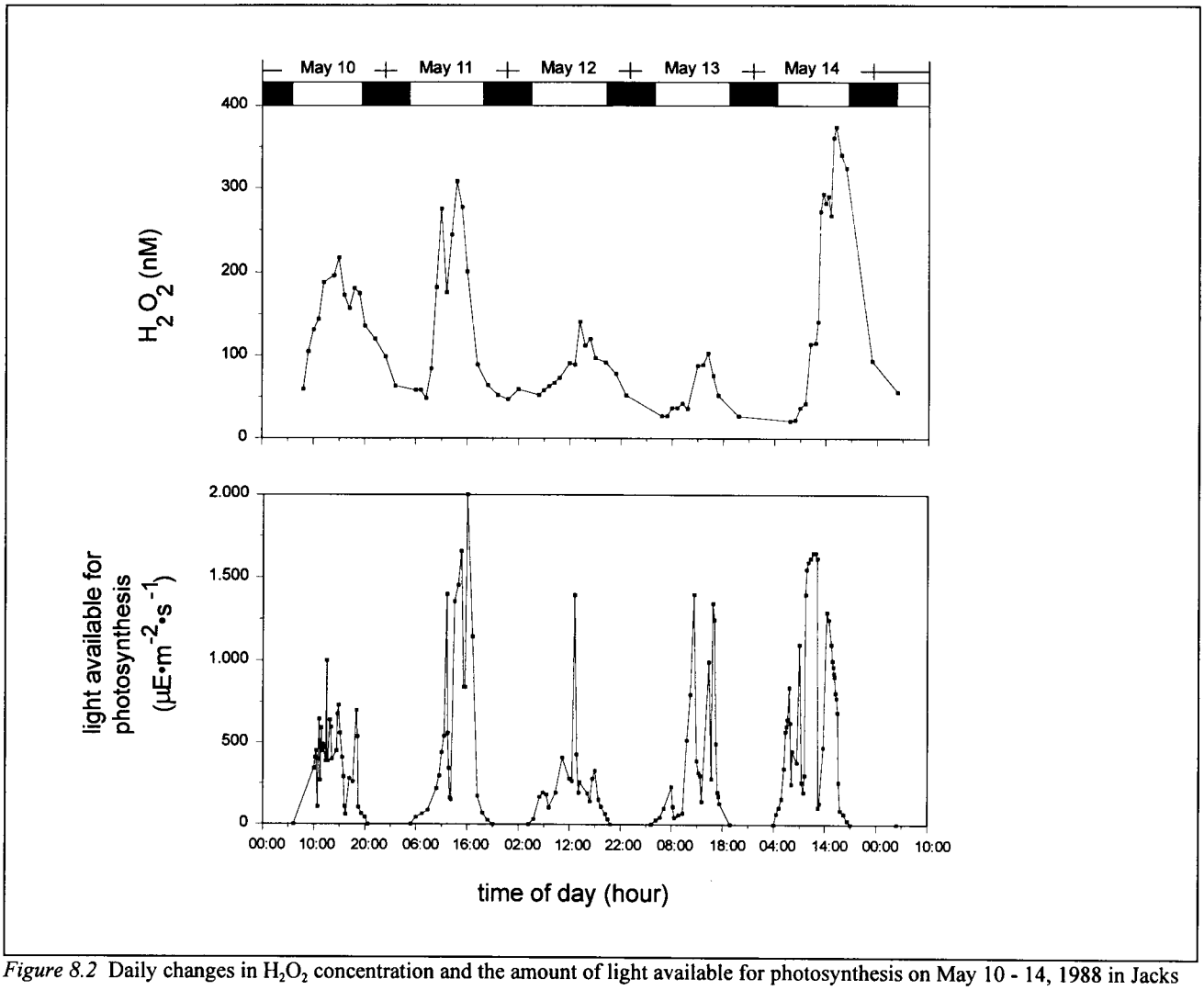

Figure 8.2 Daily changes in H_2O_2 concentration and the amount of light available for photosynthesis on May 10 - 14, 1988 in Jacks Lake in Ontario. The amount of light available for photosynthesis is given in μEinstein·m^{-2}·s^{-1}; 1 μE = 1 mol of photons. (Source: Coo89).

Dimethyl sulphide (DMS)

Dimethyl sulphide is another volatile sulphur compound that influences cloud formation. DMS is formed from dimethyl sulfoniopropionate that is used as an osmotic by marine algae (Mal92). One of the most important producers of this compound, the alga *Emiliania huxleyi*, is very sensitive to UV radiation: its growth is inhibited at an intensity as low as that corresponding to approximately 10 minutes afternoon radiation at subtropical latitudes (Han92). An increase in UV irradiation will have an effect on

DMS opposite to that on COS, and therefore also an opposite effect on cloud formation and penetration of UV in the atmosphere. There is thus a reinforcing mechanism in the case of DMS.

8.3.3 *Photochemical reduction of oxidised metal species*

The photochemical reduction of iron (Fe) and manganese (Mn) has been that best studied. The Fe- and Mn-containing compounds present in oxygen-rich water are poorly soluble and are present in colloidal form. These colloids are efficient absorbers of various compounds and materials, e.g. micropollutants such as heavy metals and pesticides. Reduction of Fe and Mn increases their solubility considerably, with consequences for their adsorbing potential to chelators. The altered solubility can also influence the availability of the adsorbed compounds and of the metals for organisms. In this way, photoreduction of Fe and Mn can increase algal growth (Mor90). The exact mechanism of the photoreductive dissolution of metal oxides is unknown.

Recent studies of the photolysis of dissolved Cu(II)-amino acid complexes have shown that these are easily broken down by UV light with formation of Cu(I) and radicals derived from the amino acid (Hay91). Photoreactivity of the CU-amino acid complexes depends strongly on the structure of the amino acid. This is mainly the result of differences in UV-absorption spectra of the complexes.

Photoreduction of metal oxides slows the sedimentation of metals in aquatic ecosystems. This effect is augmented by the photo-inhibition of biological oxidation of metals. Both photo-processes stimulate the availability of metals for primary production and can be intensified by an increase in UV radiation.

8.3.4 *Photochemical breakdown of synthetic organic molecules (pesticides, etc.) in solution and adsorbed to particles*

UV radiation induces, in addition to the conversion of natural substances such as humic compounds, amino acids and alkaloids, the breakdown of several dissolved xenobiotic chemicals (Mil90). For instance, the much-used herbicide, MCPA, is photolysed directly under the influence of UV-B. Indirect oxidation also may occur by photochemically formed OH·.

Xenobiotic molecules in aquatic ecosystems are present mainly adsorbed to particles (see also section 8.3.3). In this form their photolysis or photochemical oxidation is influenced by various physical and chemical factors such as light extinction by the particles, transport of energy over these particles, surface effects and transport to the light zone. These interactions make modelling of the photochemistry of xenobiotic compounds very difficult.

The light sensitivity of synthetic compounds generally increases with decreasing wavelength. This is related to the fact that these compounds often have their maximum absorption in the UV range. Based on this, an increase in UV radiation and a shift to shorter wavelengths will cause in increase in the breakdown of, for instance, pesticides. This applies to both direct photolysis and that mediated by OH·. As a result of this, the persistence of these compounds in the components of aquatic systems that can be reached by UV radiation will decrease.

8.3.5 *Photochemical breakdown of humic substances and DOC to microbial substrates*

Dissolved organic carbon (DOC) in rivers, lakes and oceans represents the largest supply of organic carbon on earth. The aquatic DOC pool is therefore an important component of the global carbon cycle. This pool consists mainly of humic substances that are difficult to break down. Only little is known of the fate of these compounds in aquatic ecosystems. Under the influence of UV radiation, substrates that can easily be broken down, like pyruvate, formaldehyde, acetaldehyde and glyoxylate, can be formed from humic substances (Mop90). Only UV-B radiation between 290 and 315 nm is responsible for this. Formation of OH· plays a dominant part.

The photo-breakdown of humus and the microbial breakdown of the substrates formed are probably responsible for the fact that river-supplied humus contributes little to the DOC pool in the oceans. As a result of these processes the dynamics of humus in fresh waters are also much more complex than has been assumed thus far. In eutrophic humus-rich lakes, the photo-breakdown of humus proceeds at approximately the same rate as the fixation of carbon by phytoplankton photosynthesis (Haa93).

The photochemical breakdown of DOC depends on various environmental factors, such as season, geographical latitude, time of day, water depth, water type and mixing processes. The rate of formation of low-molecular weight breakdown products is directly proportional to the light intensity at 300 nm and to the concentration of humus. The source of the humus (sea, lake or land) does not influence the rate of formation. Rates of the sunlight-induced breakdown of humus found *in situ* in lakes range from 7 to 22 mg C $m^{-2} \cdot d^{-1}$ (Haa93). The net daily production of marine phytoplankton, estimated at 0.3 g C $m^{-2} \cdot d^{-1}$, is therefore at least one order of magnitude higher than the photo-breakdown of humus. There are indications that, in seas and oceans, the photo-breakdown of carbon fixed by phytoplankton is greater (5 - 40%) than the breakdown of humus (Laa85).

The major consequence of an increase in UV radiation will therefore be increasing mineralisation of the humus pool and, as a result, increased availability of nutrients. A possibly increased cloud formation (see section 8.3.2) and decrease of bacterial

activity (Her93b) under the influence of UV radiation might be negative feedback mechanisms for this effect. Increased UV radiation would lead to a negative carbon balance in oligotrophic fens and pools during hot, bright summers.

8.4 Exposure of different aquatic ecosystems to UV radiation

An increase in UV irradiation has different implications for aquatic systems in different climatic zones. The effect is greatest in polar regions (Har89b). The aquatic environment in these regions is largely covered by a layer of ice in winter. In spring, when more light becomes available, a thick layer of algae develops on the underside of the ice, that serves as food for a complex biotic system of bacteria, protozoa and grazers such as copepods and krill. Such an ice ecosystem cannot exist without the ice algae, the photosynthesis of which is adapted to the low levels of light penetrating the ice and snow layers. Measurements have indicated that, specially under thin ice with little snow cover, wavelengths from the UV-A range are part of this light. Adequate spectral measurements that also encompass the UV-B range have not yet been performed.

In the oceans the open-water ecosystem, consisting mainly of micro-organisms, is probably adapted to high doses of UV radiation. This is the case both in the tropics, where high doses prevail throughout the year, and in the polar regions, where they occur only in spring and summer with the sun around zenith (see section 8.2). The neuston is a special aquatic system in both fresh and salt waters. It is a complex of bacteria, algae, copepods and fish larvae, all adapted to life in the upper millimeters where UV exposure is maximal. The coral reefs, that develop especially in the tropics, are a specific oceanic ecosystem that also has to be adapted to high irradiation by visible and ultraviolet light.

High UV-exposure is also possible can also occur along the borders and coasts of lakes, seas and oceans as a result of the shallowness of the water. In these locations reeds, algae, sea grasses, mangroves and their associated aquatic bottom animals are particularly irradiated, especially in the tidal zone. Mud-flat systems, like those in the Netherlands in the Wadden Sea, are found around the world. High UV doses are common in these areas, especially when the mud flats are exposed at low tide.

8.5 Effects of UV radiation on biotic and abiotic system mechanisms

As described in preceding sections, organisms that are part of aquatic ecosystems influence both each other and the abiotic environment of which they are part. On the other hand, the hydrological and physico-chemical properties of the water influence the organisms. It is therefore important to consider the consequences of irradiation by UV light with different wavelengths, not only for the biological, but also for the

abiotic components of these systems. In other words, action spectra for both biotic and abiotic processes have to be determined to find whether the expected changes in UV irradiation will result in photo-reactions that influence the system. In this section the committee describes the processes that are known to be influenced by an increase of UV irradiation over the natural levels. The last decades' literature on this subject has been summarised in several recent reviews (Kra87, Häd91, Pal91). The committee summarises these reviews and if necessary adds indications of radical changes in the system that are less obvious, little studied and therefore not dealt with in the reviews.

8.5.1 *Influence of UV radiation on key processes in aquatic systems*

Repair of DNA damage

In chapters 2 and 6 the committee discussed the different types of damage that UV radiation inflicts on DNA, as well as the mechanisms that repair this damage. The formation of thymidine dimers also has been studied in algal cells (Han94, Kar91). A recent development concerns the detection of photoproducts. There is now an immunochemical method, using an antibody that was developed in cancer research (Roz88).

Photosynthesis

The most important process in aquatic systems is primary production, the motor for all of the earth's ecosystems. Experiments with natural phytoplankton populations in the waters surrounding Antarctica showed that, during the maximum development of the ozone hole, in September/October 1990, primary production decreased by approximately twenty percent (Smi92). Since it must be assumed that, everywhere on earth, the plankton is adapted to UV levels of the period before the anthropogenic ozone depletion, these results may be extrapolated to other aquatic environments. A world-wide decrease of primary production, both aquatic and terrestrial, would of course have consequences for all ecosystems, since plant growth is the basis of the food chain and, as such, can direct interactions in ecosystems.

Variability in sensitivity; protective mechanisms

Some phytoplankton species have long been known to be more sensitive to UV radiation than others. As a result of this, species shifts might occur in communities that are exposed to increased levels of UV radiation and biodiversity could decrease. Figure 8.3 shows an example of the alterations that may occur in the populations of diatoms of the Dutch Wadden Sea under the influence of increasing UV irradiation. The

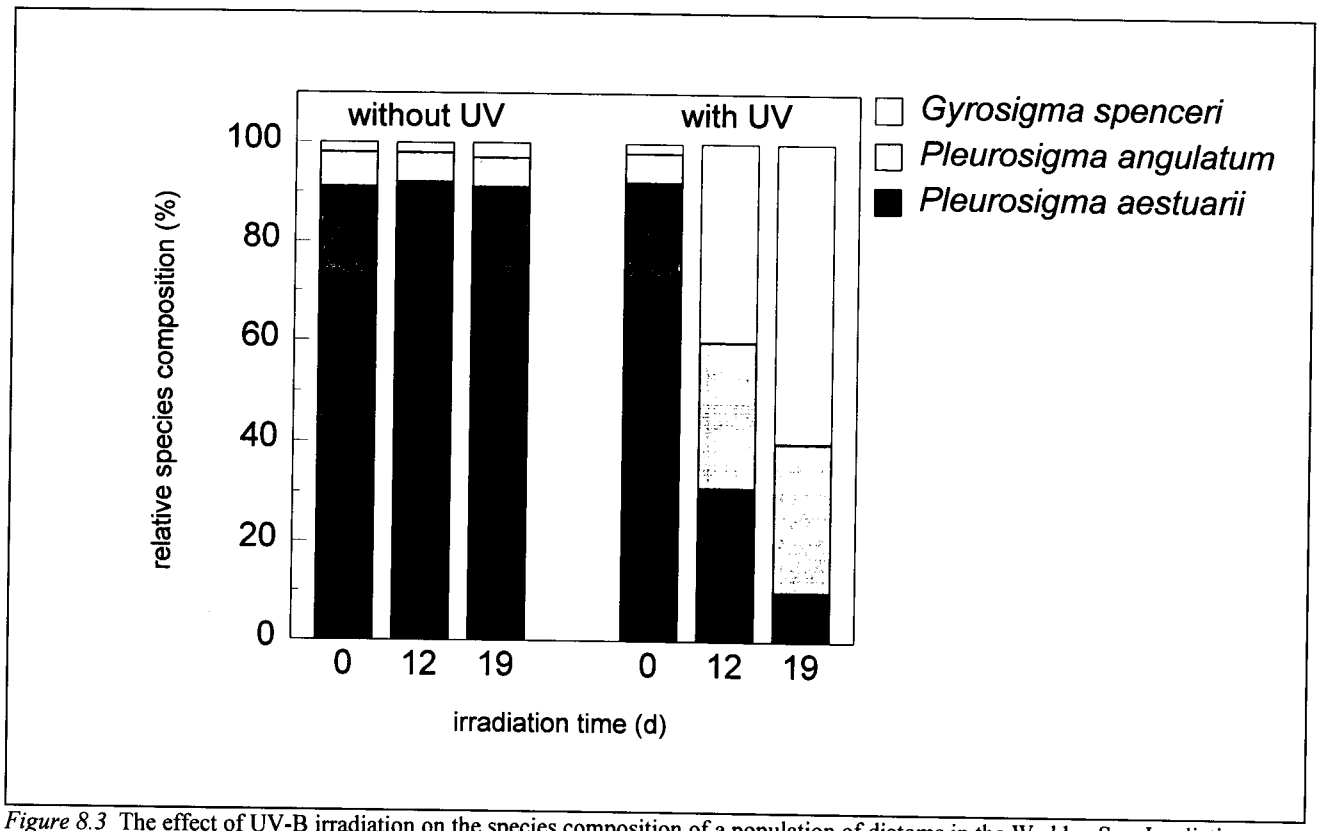

Figure 8.3 The effect of UV-B irradiation on the species composition of a population of diatoms in the Wadden Sea. Irradiation was for 19 days, 4 hours per day, with an intensity of 70 $\mu W \cdot m^{-2} \cdot s^{-1}$. The left-hand side of the figure shows control conditions, the right-hand side the effect of surplus UV-B. *Gyrosigma spenceri* becomes dominant over *Pleurosigma aestuarii*; the influence on *Pleurosigma angulatum* is less. (Source: Pel94).

present lack of information about the consequences of an increase in UV radiation so far make it impossible to predict if and how the composition of systems will change.

In many organisms the biologically effective UV dose depends on the repair mechanisms that are induced by wavelengths in the UV-A and visible range of the spectrum. Enzymes such as catalase and superoxyde-dismutase are responsible for the neutralisation of the intracellular damage caused by oxygen and hydroxyl radicals. The synthesis of these enzymes increases with increasing UV-B radiation (Les89). A number of organisms also possess morphological, physiological or biochemical properties that limit UV damage. Like terrestrial plants (see section 9.2.2), algae produce UV-absorbing pigments. Mycrosporine-like pigments were found in algae of coral reefs. Flavonoids have only been studied in sea-grasses, but not in other aquatic plants, algae and weeds.

Evasive reactions can be shown by all mobile organisms, from micro-algae up to fish (Häd91).

The effectiveness of all these protective mechanisms has to be considered in the context of the adaptation to UV radiation at the natural level, i.e. the level before the start of the ozone depletion.

Growth

The UV radiation-induced decrease of algal growth by can be a direct effect, caused for instance by damage to DNA or other vital cellular components that results in alterations in photosynthesis, growth, cell division, electron transport or membrane functions such as nutrient uptake. UV radiation can also be indirectly growth-limiting, for instance, because essential growth factors in water, e.g. vitamins, are photochemically altered and change their structure. Such indirect effects can, however, also stimulate growth. For example, it was shown in section 8.3.3 that photochemical reactions can influence the availability of iron positively (Pal91). There are indications that, in large parts of the ocean, iron is a limiting element for algal growth (Mar88b). However, the same photobiological processes can also release hydroxyl radicals that damage biomolecules.

Orientation and mobility

An effect of UV radiation that has received wide attention in the literature is its influence on the swimming behaviour and orientation of planktonic micro-organisms (Sch93a). Results obtained in the laboratory are too often and too easily extrapolated to field situations, however. In seas and oceans, disorientation of micro-organisms by UV radiation will not easily lead to alterations in vertical distribution (which might have consequences for the ecosystem), since currents and vertical mixing generally result in a homogeneous distribution in the upper water layers. It is only when a watermass has a very stable vertical structure that UV radiation will be able to influence the vertical distribution of certain species.

The influence of UV-B radiation on the vertical migration of tidal flats diatoms is currently under study by the University of Groningen. These diatoms fix bottom sediment by the formation of mucus and their altered migration could have consequences for the erosion of the tidal flats.

A number of aquatic organisms show movements that are related to the uptake of nutrients. The function of the rotation around the axis shown by dinoflagellates, for instance, is to create microturbulence that refreshes the water directly surrounding the cell. Possible disturbance of this kind of algal movements by UV radiation can induce a limitation of nutrients. No data are, however, available on this subject.

8.5.2 *Influence on the system*

Interaction with geochemical cycles

Changes in forcing functions occurring within a short period of time, such as the increase in UV radiation, cannot always be compensated for. Evolution of ecosystems is not always rapid enough. Changes in species composition of systems (see figure 8.3), where the disappearance of UV-sensitive species can lead to dominance of more resistant species, may have dramatic consequences, that are frequently difficult to predict because interactions are so complex. Several species play a very specific role. For instance, algae of the class *Prymnesiophyceae* produce the volatile sulphur compound, dimethyl sulphide (DMS), that induces formation of clouds in the atmosphere and therefore might exert negative feedback on UV damage (see section 8.3.2). The carbonate-producing algae of the class *Coccolithophoridae*, that are very sensitive to UV-B, play a dominant role in the global carbon dioxide cycle. The release of iron-containing compounds under the influence of UV radiation can induce a shift in the species composition of algae populations to larger diatoms. These sink faster than smaller algae. More organic carbon may thus reach the deep sea when UV irradiation increases, so that the ocean would become a better 'sink' for the greenhouse gas, CO_2: a positive side-effect of an increasing UV radiation. There are no data available regarding this, therefore one can only speculate about these effects.

As pointed out in section 8.3, the biogeochemical cycles of carbon and sulphur can be profoundly influenced by increased UV irradiation. This again may have implications for the global climate, for which these elements are important regulatory factors.

Food chain shifts

UV radiation can also influence the system in a more direct way: delay in cell division may result in an increase in size ('swelling') of the algae, that may then become available for other grazers (Han92). Figure 8.4 shows an example of changes in cellular size induced by UV radiation. Theoretically, this may lead to shifts in the food chain, but models to test this have not yet been developed.

8.6 Conclusions and recommendations

The effect of UV radiation on aquatic ecosystems depends greatly on the type of ecosystem and on the penetration depth of UV radiation. UV radiation can influence a number of geochemical, biophysical and biological processes. The scheme in figure

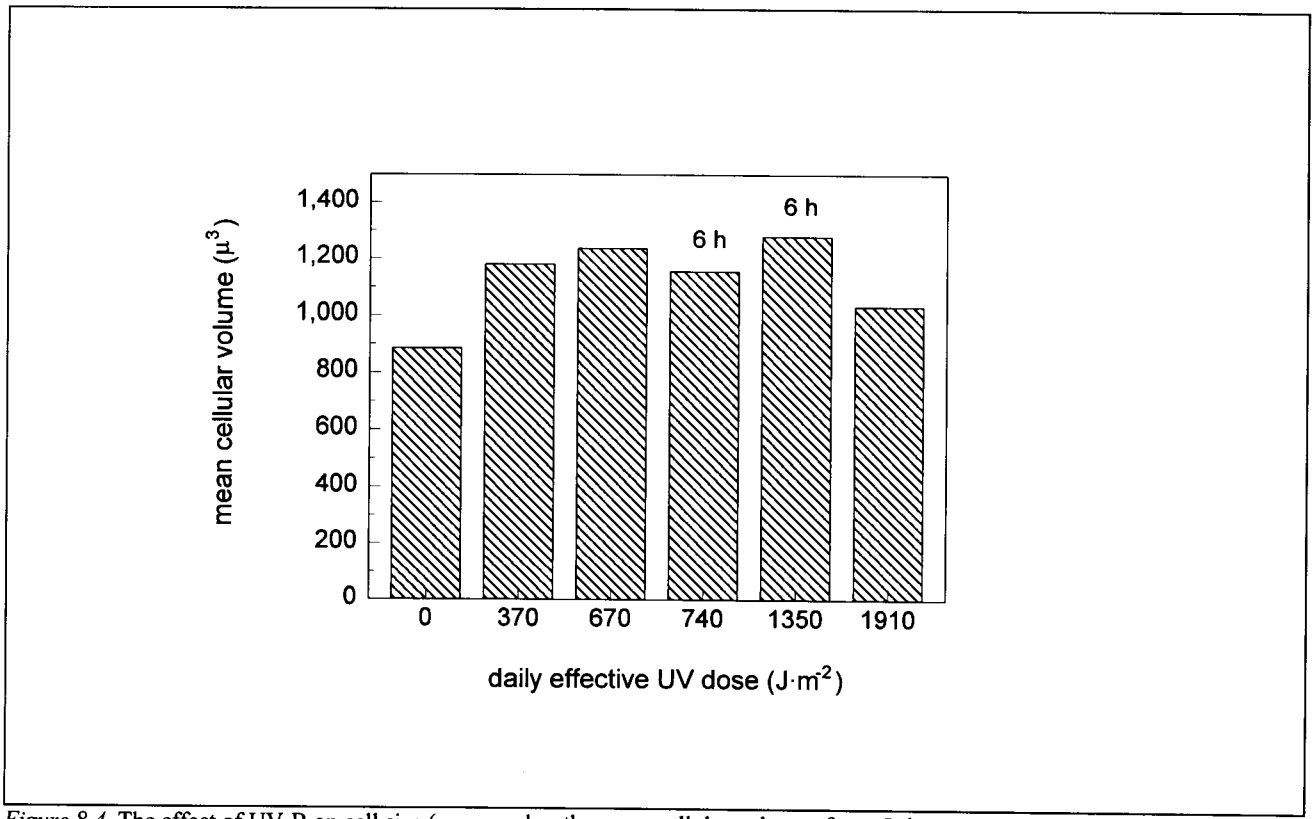

Figure 8.4 The effect of UV-B on cell size (expressed as the mean cellular volume after a 5-day exposure) of the marine diatom *Cyclotella sp.* The daily biologically effective UV-B dose (based on the general plant action spectrum of Caldwell) is given in $J \cdot m^{-2}$. The dose was applied for 3 h during the light period, except in the case of doses of 740 en 1350 $J \cdot m^{-2}$, where UV exposure was for 6 h. (Source: Bum94).

8.5 gives an overview of the relations, both proven and assumed, between UV radiation and various processes, that have been described in this chapter.

There is a great lack of information regarding all areas and organisation levels with respect to the influence of UV radiation on aquatic ecosystems. This makes it impossible as yet to predict how these systems will change with an increase in UV radiation levels. The adaptive potential of many species is probably not very high and any taxonomic variability in adaptation will lead to changes in structure and function of aquatic ecosystems. It should therefore be recommended that a number of key organisms at all levels of the food chain be selected and action spectra constructed for UV damage to those processes that are vitally important for the organisms selected. Only then will model calculations allow both the design of realistic scenarios for alterations in communities and the estimation of the risks of increasing UV irradiation.

In the sections following the committee offers several suggestions for further research.

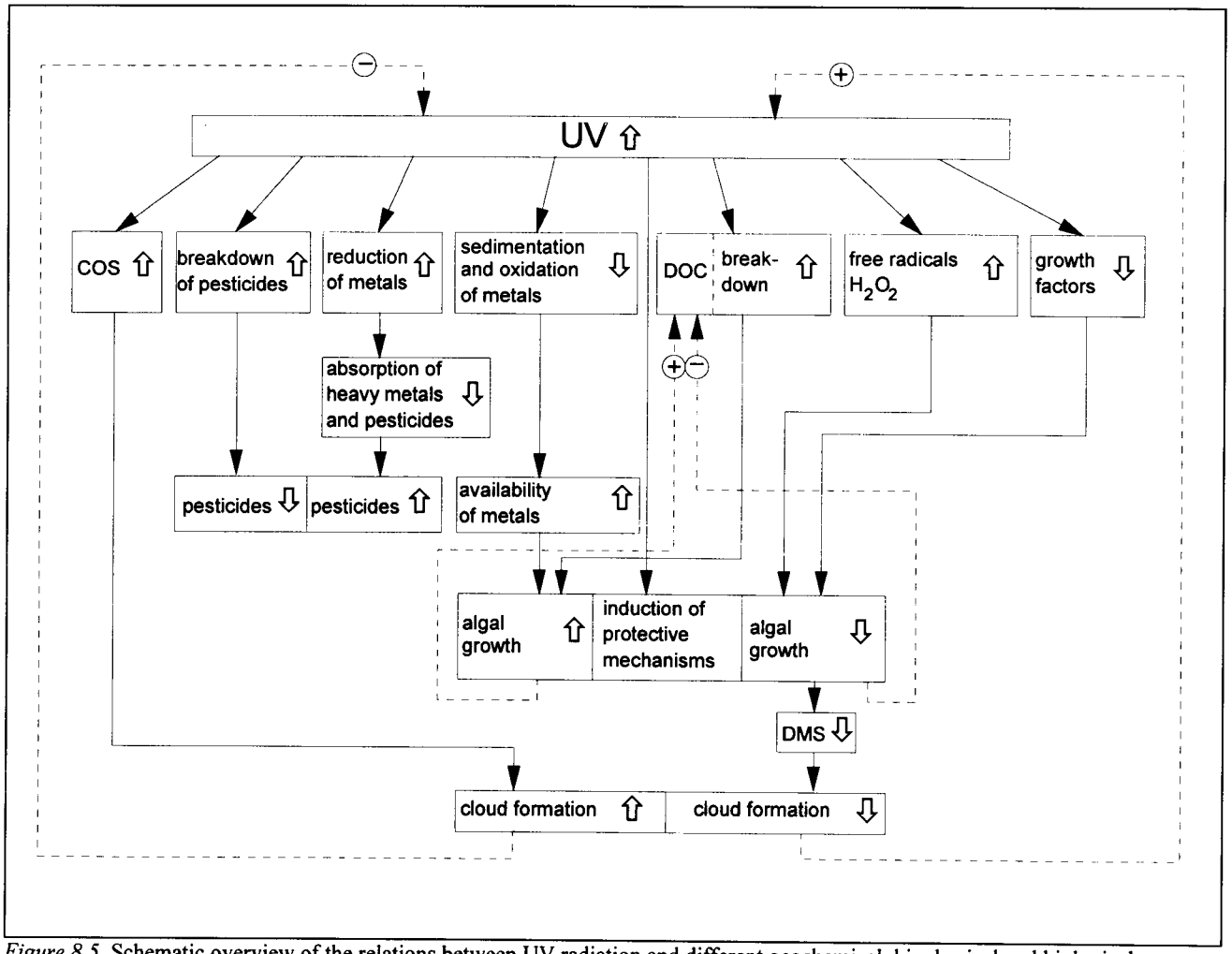

Figure 8.5 Schematic overview of the relations between UV radiation and different geochemical, biophysical and biological processes described in this report. In the boxes ⇧ indicates an increase and ⇩ indicates a decrease. In the dashed feedback loops '+' indicates a stimulating and '-' indicates an inhibiting effect. COS is carbonyl sulfide, DOC is dissolved organic carbon and DMS is dimethyl sulfide.

Light measurements

It is important to determine the full light spectrum as a function of depth in natural waters. Penetration of light in water is a function of physical, chemical, optical, biological and meteorological factors. All these factors have to be evaluated to allow a division into a number of water types, each with a certain set of these parameters.

Abiotic factors influencing the effects of UV radiation

An increase in UV radiation may influence algal growth by inducing changes in their chemical environment. These changes can be both positive and negative. Further research will have to provide more insight into the precise direct and indirect effects of an increase in UV radiation on algal growth.

In particular, more information is needed on how the results of local photochemical field studies or laboratory experiments can be extrapolated to the global scale. There is a need for data on critical photochemical processes and intermediate products, like the greenhouse gas, carbonyl sulphide (Fer84). Also, more attention should be directed to the coupling between the photochemical decay of humus and the microbial mineralisation of the decay products. Further studies also have to be performed regarding the UV sensitivity of compounds with specific ecological functions, such as vitamins, metal complexes, chelators, messengers, hormones, etc. Because of the possible regulation of primary production by 'free' metal ions a method is needed to measure the light-sensitive reactivity of metals in aquatic ecosystems.

A positive effect of an increase in UV radiation might be a reduction of the persistence in aquatic systems of xenobiotic organic compounds such as pesticides, as a result of increased photo-breakdown. On the other hand it is known that the toxicity of, e.g., polyaromatic hydrocarbons (PACs) increases with the dose of light, probably as a result of the short-wave component (Ori91). Again, not enough information is available to allow determination of the consequences of an increase in UV radiation.

Sensitivity of organisms to UV exposure

UV sensitivity is still unknown for almost all aquatic organisms. There is a pressing need for this information regarding the most important algal species of tropical, temperate and arctic waters, and for the eggs and larval stages of fish and invertebrates living in the upper water layers in these areas. Not enough is known of the vertical distribution of most organisms to allow reasonable estimates of possible exposure and damage incurred. Furthermore, almost nothing is known of the capability of organisms to adapt to UV radiation or to alterations in the UV-B, UV-A and visible light ratio, and of protection strategies, both morphological and biochemical (UV-absorbing compounds, antioxidants, stress proteins).

Modeling of UV-induced changes in ecosystems

The extrapolation of laboratory and field studies requires the development of models that include the changes in the structure of communities. These models can be based on and tested with studies on 'mesocosms' (a semi-natural environment intermediate between the monoculture in the laboratory and the completely natural field situation). However, adequate long-term studies on micro- and mesocosms that contain multiple species still have to be set up.

Determination of the consequences for global climate

The expected increase in UV radiation might have consequences for climates (see figure 8.5), an aspect that so far has received only little attention. Because phytoplankton plays a central role in the food chain, changes in its composition will have consequences for other trophic levels, such as fish and other economically important animals. However, phytoplankton also plays a central role in the biogeochemical cycles of elements such as carbon and sulphur (see figure 8.1). CO_2 is the most important greenhouse gas and a world-wide increase may lead to an increase of the average world temperature. Oceanic plants (which are 90% phytoplankton) fix CO_2 on a large scale in organic material during their growth season. Therefore, regional and temporal changes in productivity may have consequences for the fixation of CO_2 by the ocean. Some phytoplankton species are a source of DMS, a volatile sulfur-containing compound that promotes cloud formation (Mal92). Alterations in species composition may change the release of DMS to the atmosphere and thus influence the penetration of light to the earth's surface. This can have both positive and negative effects on the UV level. These effects also require further study.

Chapter 9

Effects on terrestrial ecosystems

9.1 Introduction

The effects of an increased exposition to UV radiation in terrestrial ecosystems have been studied solely with plants; there is only scarce knowledge about the effects on animals in these ecosystems (Hat94). Effects of UV radiation on plants can be found at various levels of biological organisation:

- the molecular and cellular level, where biological regulatory processes are eliminated by the experimenter,
- the organ level (leaf, pollen, flower and fruit),
- the whole-plant level,
- the population,
- communities and ecosystems.

In this chapter the committee presents an overview of present knowledge about the effects of UV exposure for each of the organisational levels of terrestrial ecosystems mentioned here. Again, none of the processes described has been investigated in such a way that any quantification is possible. This implies that it is also for terrestrial ecosystems that the committee cannot estimate the risks of an increase in UV irradiation. It is again indicated, as in previous chapters, in which areas research should be performed to allow such estimations to be done in the future.

9.2 Cellular and subcellular effects

9.2.1 *UV radiation and primary metabolism*

Of all basic physiological processes in plant cells, i.e. photosynthesis, respiration, protein synthesis and growth by cell division, the sensitivity of photosynthesis to an increase in UV irradiation has been the most investigated. Studies on isolated chloroplasts showed that UV radiation mainly influences the reaction centre of photosystem II (Iwa83, Kul83, Bor89). Increased UV exposure mostly decreases photosynthesis of intact leaves and plants (Ern93). There is however no direct relationship between decreased growth and photosynthesis.

Alterations of, or damage to biomembranes resulting from UV-B exposure have not been convincingly demonstrated, in contrast to the clearly negative effects of UV-C exposure (Mur93). The same is true for protein synthesis. Decreased growth after UV exposure must be the result of a decreased frequency of cell division, but no studies have been performed in this area.

9.2.2 *UV radiation and secondary metabolism*

Plants produce many metabolites that have no function in the primary metabolic processes of the cell: photosynthesis, respiration and protein synthesis. These secondary metabolites, however, contribute significantly to the survival of the individual and the population. They may serve to protect against abiotic environmental influences, e.g. as a selective filter against UV-B radiation, as are the flavonoids, or as deterrents to herbivores, as are the phenols, phenol derivatives and alkaloids, or to increase the resistance against pathogens, as does jasmonin acid.

9.2.3 *UV-B-absorbing metabolites*

Exposure of plants to increased UV radiation can stimulate or initiate the production of flavonoids. The accumulation of these UV-B-absorbing compounds in the cells of the epidermis protects the UV-B-sensitive chloroplasts and therefore photosynthesis. The action spectrum for the induction of flavonoids in corn and parsley has a maximum in the UV-B region (figure 9.1).

The reaction patterns of plant species with varying UV sensitivity are not consistent (Pai92). In UV-B-sensitive species the flavonoid content often increases strongly with UV-B-exposure. In UV-B-resistant species, on the other hand, all kinds of effects have been observed: an increase, a decrease or no changes in the flavonoid content.

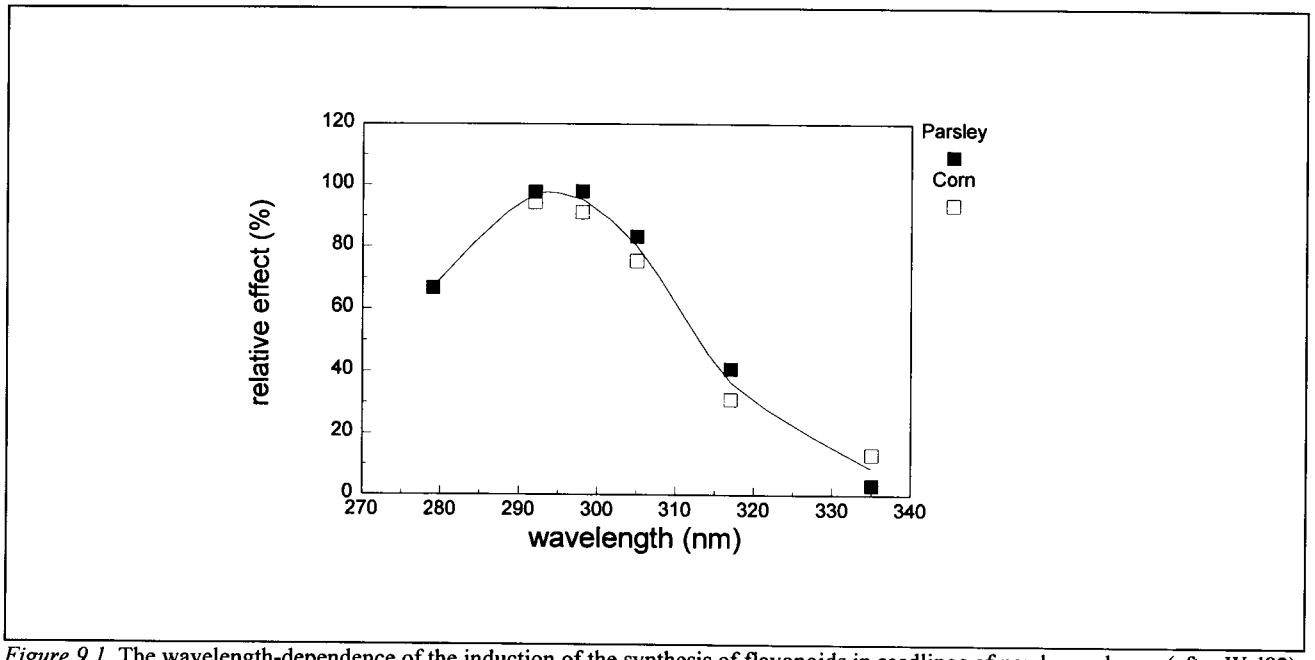

Figure 9.1 The wavelength-dependence of the induction of the synthesis of flavonoids in seedlings of parsley and corn (after Wel82).

Seedlings of UV-B-resistant barley produce more flavonoids than UV-B-sensitive corn and beans (Ter83). High-mountain plant species that are adapted to UV-B mostly do not show changes in their flavonoid content with exposure to increased UV-B irradiation (Sul92a).

Flavonoids accumulate in the epidermis of plants, thus protecting the cells of the mesophyll from adverse effects of UV-B radiation. In addition, the subcellular localisation of these compounds determines the UV-B sensitivity of the leaves.

Phenols and UV radiation

Various phenolic acids are important components of the cell walls of grasses. The physiological and biochemical properties of the cell walls are strongly influenced by these compounds. UV-B irradiation may change these phenolic acids in such a way that the cell walls lose their stretching ability. It is also possible that rearrangement of molecules in the cell wall resulting from UV irradiation decreases the breakdown of grass leaves by ruminants and delays the decomposition by soil fungi.

Modification of herbivory by alkaloids

Increased UV irradiation may affect the synthesis or the stability of alkaloids, that are important compounds in the defense against herbivores. Thus far this has only been demonstrated for the UV-B-sensitive *Aquilegia caerulea* (Lar90). Should this mechanism occur commonly, large-scale changes in vegetation might appear as a result. The lack of changes in the alkaloid content in another *Aquilegia* species and in tobacco callus cultures (Kar90) shows that the species specificity of this reaction precludes a clear unravelling of the complex plant-herbivore interactions.

9.3 Changes in organs

Both the vegetative (leaf) and the generative organs (pollen, ovary) can be affected when UV radiation levels increase.

9.3.1 Morphological alterations caused by UV radiation

Part of the UV resistance of plants is the result of UV-absorbing structures and layers on the surface of the leaf, such as hairs, wax and a silicium-rich epidermis. The number of stomata in the upper epidermis also influences the amount of UV penetrating to the deeper cell layers (figure 9.2).

In very hairy plant leaves UV absorption remains high when flavonoids and phenols are removed. Increased UV radiation may alter the structures of the epidermis, but this has only been demonstrated for the leaves of broad bean (Cal83) and *Rumex* species (Rob86).

9.3.2 Effects of UV radiation on reproductive organs

In the reproduction cycle of higher plants exposure to UV radiation can affect three phases: (1) in the pollen grain, (2) in the ovule and (3) during fertilisation. As long as the pollen is locked up in the anther, no pollen damage is to be expected because of the high flavonoid and anthocyan content of the anthers. At least 98% of UV-B is absorbed (Cal83). After release, pollen is protected against UV radiation by its high flavonoid content. At least 80% of UV-B is absorbed by the exine. The most critical phase is the germination of the pollen on the stamen. Plants that have stamens exposed to full irradiation, such as the poppy, crucifers and composites, are at highest risk. Unfortunately the effects of UV radiation on pollen vitality have only been studied in plant species that are hardly exposed to UV radiation during their life cycle. An

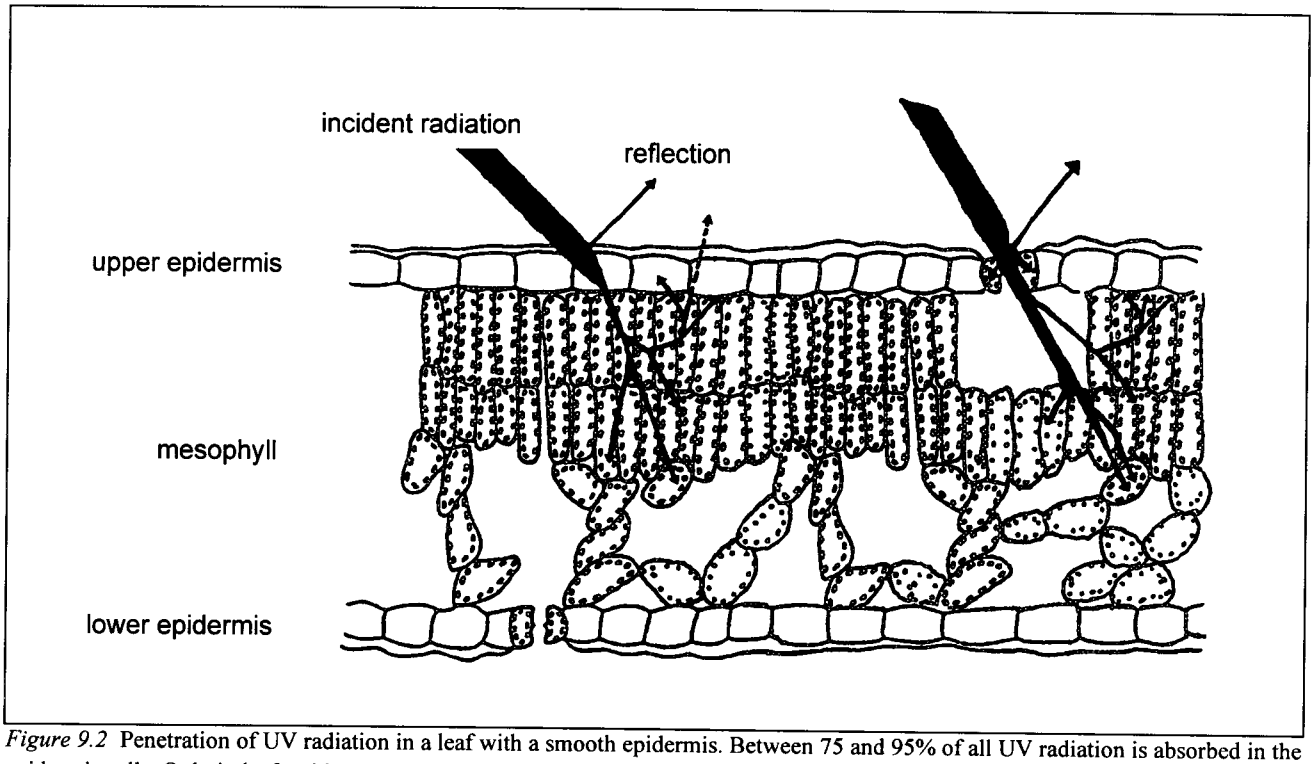

Figure 9.2 Penetration of UV radiation in a leaf with a smooth epidermis. Between 75 and 95% of all UV radiation is absorbed in the epidermis cells. Only in leafs with stomata at the upper epidermis may significant damage to the chlorophyll be inflicted locally (after Cal83, modified).

extremely high dose of UV-B inhibits the germination of pollen *in vitro,* as shown in figure 9.3 (Fli84). This figure also shows that, in the Netherlands, even with an increase in UV radiation corresponding to 20% ozone depletion no significant inhibition of pollen germination can be expected for the poppy cultivar investigated. It is not known whether this species is representative of other plant species.

No studies have been performed on the effects of UV radiation on ovules and developing embryos.

9.4 Changes at the whole-plant level

Alteration of photosynthesis and other cellular processes by exposure to UV radiation, finally, may affect the growth of the entire plant. In the field, plants are simultaneously exposed to several other abiotic and biotic factors as well. Therefore in principle only experiments lasting several years can answer the question as to UV sensitivity of a perennial plant.

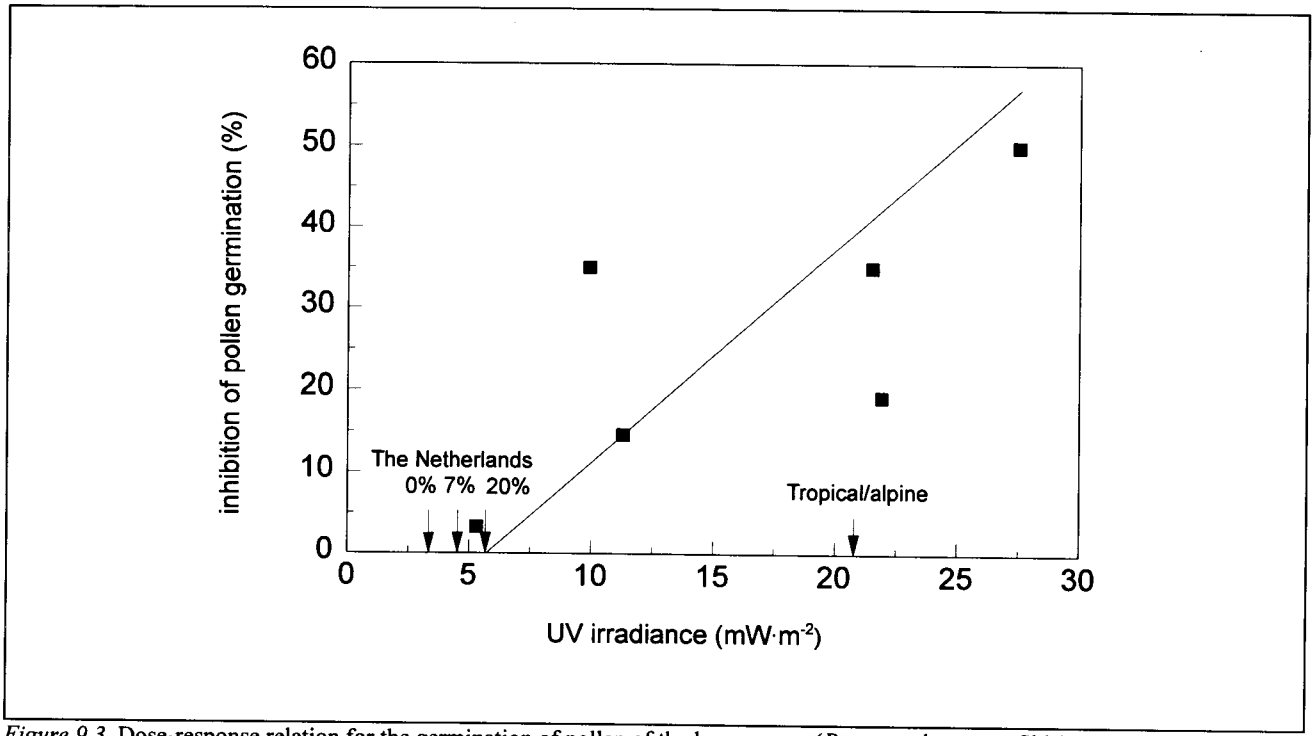

Figure 9.3 Dose-response relation for the germination of pollen of the large poppy (*Papaver rhoeas* cv. Shirley Single) after an UV-B exposure of 3 h (data of Fli84). This irradiation dose equals the maximal exposure during the summer months. The arrows indicate the irradiation levels in tropical and alpine areas and for the Netherlands, in the latter case those corresponding to an ozone depletion of 0%, 7% and 20%.

9.4.1 *Annual plants*

Many agricultural plants and pioneer species of young ecosystems (in the Netherlands, for instance, open dunes, salt marshes, woodland clearings) complete their life cycle (from seed to seed) within a year. Although greenhouse studies have shown that many agricultural plants have both UV-B-sensitive and UV-B-resistant cultivars, field experiments yielded almost no negative responses of annual crops to increased UV radiation.

9.4.2 *Perennial plants*

The complexity of the effects of increased UV exposure can be illustrated by the only long-term experiment performed so far. Four Loblolly pine (*Pinus taeda*) populations originating from regions with average UV levels varying between 8.4 and 9.7 $kJ \cdot m^{-2} \cdot d^{-1}$ (table 9.1) were exposed for three years to the natural UV radiation in Maryland (8.4 $kJ \cdot m^{-2} \cdot d^{-1}$ on average: control treatment) and to an additional 3.1 or 5.0 $kJ \cdot m^{-2} \cdot d^{-1}$ UV-B

Table 9.1 Growth reduction of *Pinus taeda* during the first three years of life, resulting from an artificial increase of the natural UV radiation (8.4 $kJ \cdot m^{-2} \cdot d^{-1}$) by 3.1 en 5.4 $kJ \cdot m^{-2} \cdot d^{-1}$, administered for 6 h daily. Significant reduction of growth relative to controls is indicated by * for $P < 0.1$ and ** for $P < 0.05$; - indicates no effect (after Sul92b, summarised).

natural UV radiation at the place of origin ($kJ \cdot m^{-2} \cdot d^{-1}$)	population	additional UV ($kJ \cdot m^{-2} \cdot d^{-1}$)	plant age (year)					
			1		2		3	
			3.1	5.4	3.1	5.4	3.1	5.4
8.4	Maryland proving grounds		-	-	-	**	**	**
8.6	Virginia		-	**	-	**	*	**
9	North Carolina		-	-	-	-	-	**
9.6	Georgia		*	**	**	*	-	*

radiation for 6 hours daily (Sul92b). Growth reduction (above and below ground biomass combined) caused by the additional UV radiation depends on the population (table 9.1); for instance the insensitive North Carolina population can be compared to the sensitive Virginia population. The age of the plant is also a factor (development-effect).

The Georgia population, of all test populations the one that originates from the region with the highest background level of UV, was very sensitive to increased UV in the first year. In the second year the sensitivity remained, but the plants treated with the lower UV level were more sensitive than those treated with the higher level. In the third year only the plants treated with the higher level showed a slight reduction in growth. On the other hand, growth of plants from the Maryland population was stimulated by the higher UV level in the first year, inhibited by the same extra UV dose in the second year, while in the third year growth was inhibited by both the lower and the higher UV exposition.

It is clear that these data do not allow predictions to be made as to the quantification of UV-induced changes in plant growth in general.

9.4.3 *Impact of other environmental factors on UV sensitivity*

Increase in CO_2

Simultaneous exposure of plants to increased CO_2 and UV-B radiation can decrease or even nullify the growth stimulation by CO_2. In principle, an increased CO_2 level decreases the effect of UV radiation on plants (Ste93b, Ter90).

Nutrients

Lack of phosphate or of phosphate and nitrogen counteracts the negative effects of UV radiation (Ern93). This may be caused by stimulation of secondary metabolism by deprivation of these nutrients (Mur85). Eutrofication of ecosystems by deposition of ammonia has an adverse effect. Ammonia raises UV sensitivity of plants, because it inhibits the synthesis of flavonoids and phenols.

Water

An insufficient water supply in combination with increased UV exposure stimulates the synthesis of flavonoids and phenols (Mur86) and often increases the production of cuticular waxes. This results in a decreased penetration of UV radiation in the chlorophyll-containing leaf cells. This reaction pattern is not found in species that are sensitive to desiccation. It can be expected that, in these species, water stress and UV stress will have an additive effect.

9.5 Effects on populations

9.5.1 *Agro-ecosystems*

Field ecosystems

In growing agricultural crops, maximisation of the yield is aimed at. According to the laws of thermodynamics the increased yield can only be realised through a loss of other energy-consuming metabolites, often compounds functioning in the defense against pests and diseases. This explains the sensitivity of many crops to pests and diseases and the need for pesticide use. The great variation in UV sensitivity of agricultural crops results from unintentional co-selection.

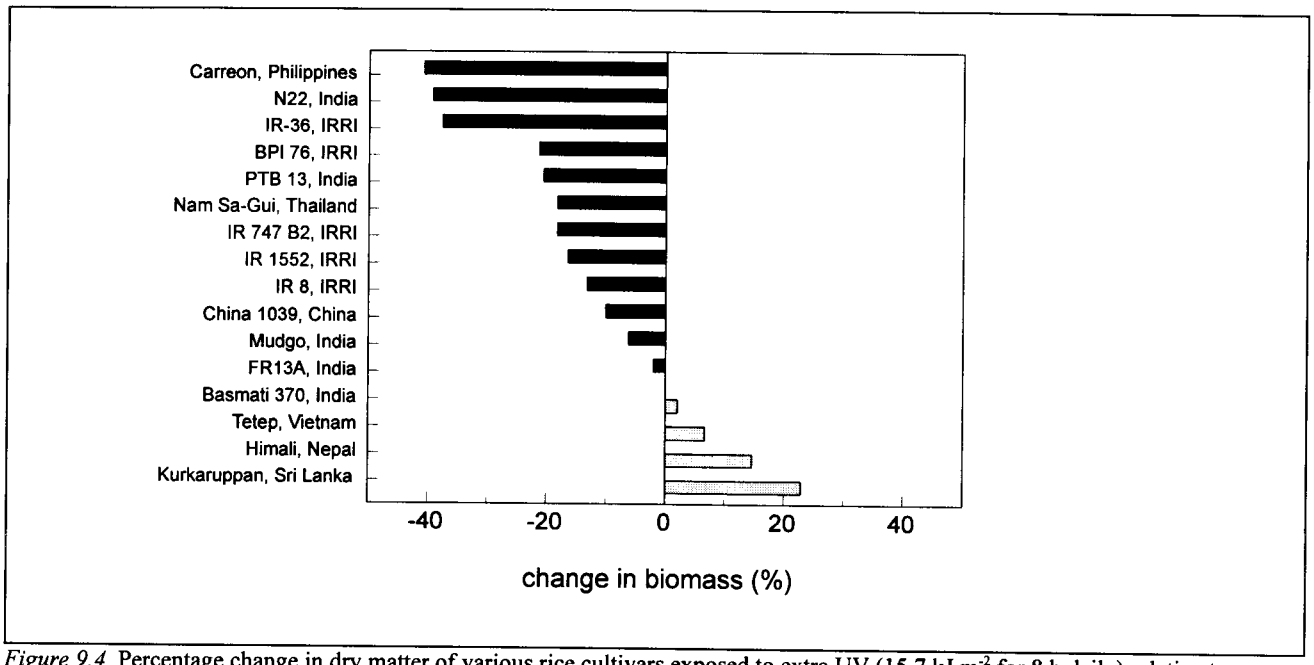

Figure 9.4 Percentage change in dry matter of various rice cultivars exposed to extra UV (15.7 $kJ \cdot m^{-2}$ for 8 h daily) relative to controls (Source: Ter91). The country of origin is indicated for each cultivar. The ones marked 'IRRI' were developed by the International Rice Research Institute in the Philippines.

Rice (*Oriza sativa*) can serve as an example. A large number of cultivars have been studied for UV-B sensitivity (Ter91). Both inhibition and stimulation of growth, as well as no effect were found (figure 9.4). These data are consistent with the just mentioned selection principle. Cultivars originating from mountain areas (600 - 2700 m, Nepal) and from the subtropical coast (6° - 15° North, Sri Lanka, Vietnam) are very tolerant. The cultivars that were developed for high yield by the International Rice Research Institute (IRRI) in the Philippines are the most sensitive (figure 9.4).

It can be assumed that there is a vast genetic variability in all agricultural crops that allows the development of UV-resistant cultivars. Therefore an increase in UV irradiation does not *per se* have to be a limiting factor for local food production.

The rate of selection can be limiting for UV-resistant cultivars that are to be developed, since it is not yet known which physiological and morphological factors are responsible for resistance. The studies with rice cultivars show that neither the increased flavonoid synthesis, nor specific changes at the leaf surface are suitable characteristics for estimation of the sensitivity for UV-B radiation.

Grassland ecosystems

Very few studies have been performed on cultivated grasses. In grasses, in contrast to the herbs found in grasslands, a UV-induced change in the binding of phenylpropanes in the cell wall does not seem to have any consequences, since the leaf elongation occurs in the basal zone that is protected by the leaf sheath. Based on the results of available studies it cannot be judged whether the possibly altered chemical structure of cell walls as a result of increased UV irradiation has a negative influence on the digestion process in animals. In view of the great variation in bacterial endo- and exohydrolases this, however, does not seem very likely.

9.5.2 *Natural and semi-natural ecosystems*

Forest ecosystems

It has already been stated in the introduction to this chapter that the risk of exposure plays an important role in the determination of the effects of increased UV radiation for organisms in natural and semi-natural ecosystems. The species that are the most sensitive to UV can be expected in ecosystems that are seldom or never exposed to significant amounts of UV radiation. These species include grasses, herbs, lichens and mosses of deciduous and coniferous forests that either complete their life cycle during the leafless period in spring when there is almost no UV radiation, or that grow under the protective canopy of trees.

Seedlings of all tree species grow primarily under the protection of the mature trees, therefore negative reactions due to exposure to UV radiation are not expected (Ern93). It is less easy to predict how adult trees will react to an increased UV flux. It is imaginable that the survival of these species in the Mediterranean area during the last ice-age has led to selection for UV resistance. This resistance might only be expressed in full-grown and therefore UV-exposed individuals (differential gene activity). On the other hand some tree species already had reached their population optimum in the post-glacial period. As a result of the dramatic decrease in population size (e.g. Scotch pine in Central Europe) the genetic potential in these species may have been considerably reduced and, as a consequence, the possibility of adaptation to changing environmental circumstances. This is also the case for cloned species such as the poplar and the elm. As with herbs and grasses, individual genetic variability in UV sensitivity must be taken into account with trees also, as seen from the presence of UV-tolerant and UV-sensitive seedlings of maple (Bog82). Generalisations about sensitivity cannot be made if nothing is known about the origin of the seed.

Damage by other environmental factors, e.g. SO_2, NH_4, NO_x and O_3, to the crowns of trees results in more UV radiation reaching the herbs and grass layer of forests. Therefore the risk of UV damage to plants and animals living in these layers may increase in forests with a damaged canopy.

As a result of the openness of coniferous forests the undergrowth is more exposed to UV radiation than it is in deciduous and other forests. The undergrowth in coniferous forests (bilberries, cowberries and heather) is primarily of high-montane or subalpine origin, therefore some UV resistance can be expected. Populations of these species that have grown in deciduous forests for millennia probably will not show this resistance (Kos81).

It can be expected that all forest animals are sensitive to UV radiation, because they are never exposed to it. Of all organisms living above ground - large mammals and birds excepted - only the herbivores living in the crowns of trees can be expected to be exposed to UV radiation, for instance larval stages of butterflies, beetles and hymenopterans. Their feeding patterns, however, are often restricted to the dark or to daytime periods with high air humidity, i.e., periods with no or very low UV radiation. Exposure to UV radiation is perhaps only obvious for sucking insects such as aphids, because these are active in the daytime and on the exposed side of leaves. No studies on this topic have been published, however.

Open semi-natural (chalk and *Molinia* grasslands) and natural ecosystems

Almost nothing is known of the reactions to UV exposure of species from open ecosystems. The UV sensitivity of plant species in a number of open ecosystems with a well-defined population differentiation with respect to abiotic factors cannot be judged from the exposure of a randomly chosen, undefined population. Because of this, competition experiments in which plant species originating from different ecosystems are exposed to UV radiation only are of value, when populations with identical ecological background are compared. There is little evidence that this has been done, however.

Comparison of populations of Bladder campion (*Silene vulgaris*, a perennial herb from (semi)natural grasslands) originating from coastal areas, from inland areas and from high mountains showed that all populations are equally resistant to UV radiation. Experiments with UV exposure of plant species from open ecosystems showed that grasses in coastal ecosystems (dunes, salt marshes) and savanna ecosystems are relatively insensitive (Ern93, Sta90). Perennial herbs are either insensitive (dune populations of *Verbascum thapsus* and *Plantago lanceolata*; Pai92) or sensitive, like the Sea-aster (Sat90). It is still too early to draw general conclusions from these data or to make predictions.

Other factors also may have an influence on the composition of ecosystems. An increase in the CO_2 content, for instance, may result in shifts in the competition and therefore in changes in the species composition of ecosystems and a decrease in biodiversity.

9.6 Conclusions and recommendations

UV-sensitive plant cultivars and species show decreased growth when exposed to the increased UV levels that are expected to result from 20% ozone depletion. Almost no or no effects can be measured after exposure to increased UV levels corresponding to 10% ozone depletion. Other environmental factors, such as the amount of photosynthetically active radiation and the supply of water and nutrients can diminish or augment the negative UV effects.

Insufficient knowledge is available about the effects of UV radiation on terrestrial species, populations and ecosystems to allow predictions as to the effects of an increase in UV irradiation, let alone quantification of these effects. Based on general ecological patterns, the genetic variation in UV sensitivity present at the species level (populations, cultivars, races), uncertainty as to the rate of evolution of UV resistance and the diversity of exposure of species in ecosystems and between ecosystems, it can be expected that a fast increase in exposure to UV radiation may have negative consequences for components of ecosystems or for entire ecosystems. This can be the case especially when other environmental factors increase UV exposure or inhibit the synthesis of protective compounds. This also applies to ecosystems consisting of dominant species in which genetic variation has been reduced by human action, i.e., agricultural crops.

In order to gain more insight into the influence of UV radiation, especially on ecosystems in the Netherlands, field studies should be performed to:

- determine the sensitivity of crops in the Netherlands over the entire growing season,
- measure the sensitivity of species in (semi)natural open ecosystems and determine the potential shifts in species composition,
- measure the potentially cumulative effects of UV radiation in long-term experiments on perennial grasses and herbs, shrubs and trees,
- determine the consequences for herbivores of changes in the chemical composition (phenolic acids, flavonoids, alkaloids) of leaf material,
- analyse the sensitivity of the different life stages of leaf- and flower-consuming insects.

Chapter 10

Preventive measures

Sunlight is an essential factor for life on earth. The photosynthesis-driven formation of organic material by plants is at the basis of almost all food chains. Sunlight is also important for humans. The most important way humans take in information from the environment is by vision. Vision starts with photochemical processes in the retina. Sunlight is also important for physical health. Vitamin D_3 is synthesised under the influence of the UV component of sunlight. Also, exposure to sunlight and the warmth of the sun promotes well-being. A tanned skin is still an ideal in several western countries.

It is not always realised that exposure to sunlight when sunbathing also presents risks, primarily from UV radiation that reaches the skin and the eyes. In preceding chapters the committee has explained that skin cancer, ageing of the skin, eye disorders and suppression of the immune system may occur. It is clear that excessive UV exposure should be avoided in order to minimise as far as possible the risk or the rate of development of such disorders. The increase in UV irradiation that is expected in coming decades and that is already measurable in some places, will lead to a higher incidence of these lesions, assuming that other circumstances, and especially behaviour, remain unaltered.

Plants and animals can also be influenced by the increased UV irradiation. During evolution, they have adapted to a certain level of UV radiation and the question is whether they can adjust rapidly enough to further changes in this level. Should this is not be the case, there may, as described in preceding chapters, be a number of far-

reaching consequences possibly contributing to changes in the global climate. There are thus a number of reasons to aim at rectifying the increase in UV irradiation.

The effects of an increase in UV irradiation on humans and those on (components of) ecosystems constitute an added risk. Part of the environmental policy of the government of the Netherlands is to limit added risks, as described in section 1.2. In order to achieve this, the government applies source-directed and effect-directed measures. In the present chapter the committee presents several recommendations for each of these groups of measures.

10.1 Measures at the source

In the case of UV irradiation, reversing the increase in UV irradiation occurring as a result of ozone depletion is the only feasible source-directed measures. The only way this can be done is by halting the emission of substances that affect the ozone layer.

10.2 Limitation of exposure

Limitation of exposure is virtually impossible in the case of natural ecosystems, but can be achieved in a number of ways for humans. Imposing measures to limit sunlight exposure is out of the question in a democratic society. The government can only undertake to provide adequate information.

10.2.1 Protection of the skin against sunlight

From the point of view of reducing risks it would be best to avoid sunbathing completely. However, the zest for sunbathing is such that it occurs on a large scale. Accepting that this is reality, it becomes essential to provide education about risks and how to reduce them. This education should aim to promote moderation, and certainly avoidance of sunburn, especially in children. The use of sunbeds and other artificial UV sources should also be covered by this information. The Health Council has addressed the health effects of the use of these devices in a previous report (GR86). Unintentional sunlight exposure in daily life contribute greatly to the risk and should therefore also be covered by the information.

Protection of the skin against sunlight is an important method of primary and also possibly secondary prevention. The initial effect of primary prevention measures, e.g. education, will be a change in attitude and behaviour of the population. Educational campaigns have been specially directed at sunburn prevention and us of sunscreens. In Australia, education of this type has resulted in a change in behaviour of the population. An effect on the incidence of skin cancer or eye disorders will only appear later.

In the case of the development of melanomas there are early indications that specific education and information campaigns are effective (Gir91, McK92b).

10.2.2 Avoidance

Avoiding sunlight is especially effective during the summer months between 11.00 and 15.00. Sunbathing in this period should be strongly discouraged.

10.2.3 Sunscreens

The use of sunscreens was initially considered a good means to prevent carcinomas. There are recent indications, however, that sunscreens protect adequately against UV-induced development of erythema, but that it is still unclear to what extent they can also prevent long-term effects such as skin ageing and the development of skin cancer. The sunscreen protection factor, based on the prevention of erythema formation, probably has a different, lower, value for effects such as DNA damage, immunosuppression and skin ageing (Pra91, Pra93, Wol93b).

The committee concludes that it is undesirable to use sunscreens to allow a longer stay in the sun than would be deemed possible without this protection. The committee also points out that incidental non-use of sunscreens results in a considerable increase in the risk of adverse effects, since the development of the most important natural protection against UV radiation, thickening of the skin, is considerably reduced when sunscreens are used. Moreover it is also important for prevention of effects other than sunburn that sunscreen be applied to all exposed skin areas, including those that do not burn easily, e.g. the skin of the head and hands.

10.2.4 Pigment

Natural pigment offers good protection against the formation of erythema and against carcinogenesis. This is especially the case for the pigment present in the dark skin. The pigment that is formed under the influence of UV radiation has some protective potential, but its synthesis is preceded by infliction of damage. Neither of these pigments is, however , thought to protect against immunosuppression (Ver91).

10.2.5 Clothing

The skin can also be protected against sunlight by clothing. It should be realised that some clothing offers better protection than do other types. In Australia certain clothing is therefore given a protection factor (Jev90). Wearing a broad-rimmed hat is very

effective to reduce the UV dose to the skin of the head and neck and to the eyes, areas with at greatest risks of long-term effects.

10.2.6 *Protection of the eyes*

Traumatic eye disorders that are caused by UV radiation, e.g. welders' eyes and snow-blindness, both acute corneal inflammations, can be prevented if sufficient protective measures are taken. For welders, these consist in wearing adequate protective devices, such as welding goggles. It is recommended that adequate, no UV-transmitting snow-glasses should be worn for winter sports. These should also offer effective shielding at the bottom and sides, since a considerable portion of the UV irradiation in snow-covered areas results from reflected UV radiation (fresh snow reflects more than 80% of the incident radiation) (Sli86).

In chapter 7 the committee indicated that UV radiation possibly has an effect on the formation or development of cortical and posterior subcapsular cataract. In order to reduce the influence of UV radiation on these or other eye disorders it should also be recommended that the eyes should be shielded as well as possible from UV radiation. The amount of direct radiation can be reduced considerably by the wearing of a hat or a cap with a brim or a visor on the front. Sunglasses can also be effective, provided the glass does not transmit UV radiation; if it does, glasses can have the opposite effect. Because of a reduction in the intensity of visible light, the pupil will dilate and more UV radiation can reach the lens (Kui91, Sil86). Even sunglasses that fully block UV radiation cannot completely protect the lens. Reflection can result in considerable amounts of UV radiation reaching the eye from the sides and bottom (Jav91, Sli80). This is especially the case at or near reflecting surfaces such as water and light sand (Sli86). In such cases use of sunglasses that offer adequate shielding on the sides and on the bottom, as do snowgoggles, should be recommended. The effects of head coverings and sunglasses are additive. If both are worn, UV exposure can be reduced to approximately 90%.

10.2.7 *Final remark*

In view of the Australian experience, the committee expects that, given adequate education and information, it is possible to bring about a change in the behaviour of the population. This might more than compensate the effects on humans of an increase in UV irradiation resulting from ozone layer depletion.

The Hague, June 2, 1994,
for the committee,
(signed)

Dr E van Rongen
secretary

Professor Dr JC van der Leun
chairman

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Annexes

Annex **A**

The request for advice

In a letter dated May 19, 1992, the Dutch Minister of Housing, Spatial Planning and the Environment requested the Health Council for advice on the risks to humans and ecosystems resulting from exposure to ultraviolet radiation. The request for advice was formulated as follows:

The depletion of the ozone layer causes world-wide concern. Actions to limit among others the production of CFCs (Montreal Protocol) express these concerns. Recent scientific views indicate that there is a clear relation between depletion of the stratospheric ozone layer and the increase in ultraviolet radiation in the upper atmosphere. An increase of particularly UV(B) radiation in the biosphere may lead to higher exposure and in this way to an increase in health damage caused by UV radiation. Recent reports on possible significant changes in the stratospheric ozone layer over the Northern Hemisphere, and the possible associated health risks, have caused increasing concern in the Dutch population.

In a previous report (1986) the Health Council described the effects on health that may occur in humans as a result of exposure to UV radiation. Furthermore this report examined the possibility of regulating the unwanted negative effects of exposure to artificial sources of UV radiation (e.g. sunbeds). Since then research has been performed world-wide on the relation between exposure to UV radiation and the occurrence of negative health effects such as, for instance, the incidence of skin cancer.

UNEP recently published a report on environmental effects of ozone depletion that presents new data on the adverse effects on humans, materials and ecosystems (Environmental Effects of Ozone Depletion: 1991 Update, November 1991).

The Dutch National Institute of Public Health and Environmental Protection (RIVM) also issued a report in 1991 that attempted to quantify the adverse human health effects resulting from exposure to UV

radiation as an additional risk subsequent to human actions (report nr. 249104002, Blootstelling aan ultraviolette straling, Slaper and Eggink, October 1991). Although there are a number of uncertainties associated with this attempt to quantify the risk of increased UV exposure, it clearly shows that, based on a comparison with other environmental risks, this possible increase in UV radiation in the outdoor environment and the associated negative environmental effects warrant high political priority. This leads to questions concerning the quantification of effects resulting from exposure to UV radiation. I would therefore like, also in view of the results of the above-mentioned reports, to ask the advice of the Health Council regarding the following five items.

1 Have there been new scientific developments since the publication of the 1986 advisory report that more clearly relate exposure to UV radiation and effects on the human immune system? Is it possible to translate such effects, e.g. immunosuppression and the related increase in, for instance, infections if they can be demonstrated into dose-effect relations for diseases or mortality? If such dose-effect relations can be quantified, what is the risk of mortality and/or incidence of disease?

2 Many model calculations show a relation between depletion of the ozone layer and a certain increase in UV radiation in the biosphere, resulting in an increase in the incidence of non-melanoma skin cancer. Have new views been developed concerning the value of the risk factor that relates this cancer incidence and exposure to UV radiation? The same question can be asked with respect to the quantitative relation between mortality resulting from skin cancer and the incidence of cancer resulting from exposure to UV radiation.

3 Until recently it was not clear whether UV radiation may also (partly) be responsible for the induction or stimulation of the development of melanomas. Is there at this time, based on recent scientific developments, more certainty about the relation between UV radiation and melanoma incidence, especially about the possible role of UV radiation as a co-factor? Can such relations be quantified? Are there any new developments that are important with respect to determination of the mortality risk associated with melanomas?

4 Have sufficient indications become available to show that more eye disorders than previously assumed can be related to exposure to UV radiation? Is it possible to give so-called risk numbers based on existing and new data on UV exposure-induced eye diseases?

5 In the past, research related to the effects of UV radiation has been primarily aimed at effects in humans. Increasingly, questions are asked about the possible negative effects of UV radiation on ecosystems. Has scientific knowledge already advanced in such a way that possible negative effects on ecosystems, for instance aquatic ecosystems, can be quantified? Is the system for handling effects in ecosystems, that has been presented in the publication 'Dealing with risks' (1989) of any help for performing such quantifications?

(signed)
The Dutch Minister of Housing, Spatial Planning and the Environment
JGM Alders

Annex **B**

The committee

On September 17, 1992, the President of the Health Council installed the committee 'Risks of UV radiation'. The makeup of the committee is as follows:

- Professor Dr JC van der Leun, *chairman*
  emeritus professor of Dermatology, University of Utrecht
- Professor Dr W Admiraal
  professor of Aquatic Ecotoxicology, University of Amsterdam
- Professor Dr WHO Ernst
  professor of Botany, Free University, Amsterdam
- Dr WWC Gieskes
  oceanographer, University of Groningen
- Dr FR de Gruijl
  biophysicist, University of Utrecht
- Dr H de Haan
  limnologist, Department of Water Management, Province of Friesland, Leeuwarden
- Dr DWG Jung, *advisor*
  physicist, Ministry of Housing, Spatial Planning and the Environment, Den Haag
- Dr RJ van Kempen, *advisor*
  radiation specialist, Ministry of Welfare, Public Health and Culture, Rijswijk

- Dr H van Loveren
  immuno-toxicologist, National Institute of Public Health and Environmental Protection, Bilthoven
- Dr WF Passchier, *advisor*
  physical chemist, Health Council, Den Haag
- Dr L Roza
  biochemist, TNO Food, Rijswijk
- Dr H Slaper, *advisor*
  biophysicist, National Institute of Public Health and Environmental Protection, Bilthoven
- Professor Dr KEWP Tan
  professor of Ophthalmology, Free University, Amsterdam
- Professor Dr BJ Vermeer
  professor of Dermatology, University of Leiden
- Professor Dr GFJM Vrensen
  professor of Ophthalmo-morphology, University of Leiden and Netherlands Ophthalmic Research Institute, Amsterdam
- Dr JH Vosjan
  microbiologist, Netherlands Institute for Sea Research, Den Burg, Texel
- Professor Dr AA van Zeeland
  professor of Molecular Radiation Dosimetry and Radiation Mutagenesis, University of Leiden
- Dr E van Rongen, *secretary*
  radiobiologist, Health Council, Den Haag

Following the founding meeting the committee continued its work as two working groups, the 'Effects on humans' group and the 'Effects on ecosystems' group. After completion of the work of these working groups the committee convened once in full.

The 'Effects on humans' working group met fourteen times. Its composition was as follows:

- Professor Dr JC van der Leun, *chairman*
- Dr H van Loveren, *vice-chairman*
- Dr FR de Gruijl
- Dr DWG Jung, *advisor*
- Dr RJ van Kempen, *advisor*
- Dr WF Passchier, *advisor*
- Dr L Roza

- Dr H Slaper, *advisor*
- Professor Dr KEWP Tan
- Professor Dr BJ Vermeer
- Professor Dr GFJM Vrensen
- Professor Dr AA van Zeeland
- Dr E van Rongen, *secretary*

The 'Effects on ecosystems' working group met four times. Its composition was as follows:

- Professor Dr JC van der Leun, *chairman*
- Professor Dr W Admiraal, *vice-chairman*
- Professor Dr WHO Ernst
- Dr WWC Gieskes
- Dr H de Haan
- Dr DWG Jung, *advisor*
- Dr RJ van Kempen, *advisor*
- Dr WF Passchier, *advisor*
- Dr JH Vosjan
- Dr E van Rongen, *secretary*

Administrative assistance was given by mrs R Aksel-Gauri and editorial support by Dr AB Leussink, both of the Health Council Secretariat.

Annex C

Report of the Workshop

Introduction and background to the workshop

There is an ongoing debate concerning the potential health effects of depletion of the ozone layer and the concomitant increase in ultraviolet (UV) radiation in the biosphere. The Dutch Government is concerned about the public health impact of this particular environmental degradation and charged the Dutch Health Council with delivering advice about the prevention or mitigation of human health damage related to the expected UV increase. While there is agreement that UV irradiation is related to skin cancer, there are conflicting opinions concerning the impact of UV radiation on the ocular lens. Some scientists feel that UV is a causative factor in lens opacification; others feel that the evidence supporting a role in cataract is weak. In order to clarify the role, if any, of UV radiation in cataract, the Dutch Health Council elected to host a specialist workshop of experts in the fields of epidemiology, lenticular/corneal ophthalmology, biochemistry, photochemistry, photobiology, pathology, toxicology and risk assessment. This workshop, entitled '*The UV Scenario for Senile Cataract: Fact or Fiction ?*', was held in Rotterdam, The Netherlands, January 31 - February 1, 1994.

Participants

The following international experts were present at the workshop as active lecturing participants:

- James P Dillon, Columbia University, New York, NY, USA

- Paul Dolin, University of London, London, UK
- John W Eaton, Albany Medical College, Albany, NY, USA
- John J Harding, University of Oxford, Oxford, UK
- Kenneth R Hightower, Oakland University, Rochester, MI, USA
- Otto Hockwin, St Augustin, Germany
- Jonathan C Javitt, Georgetown University Medical Center, Washington, DC, USA
- Barbara EK Klein, University of Wisconsin Medical School, Madison, WI, USA
- Masami Kojima, Kanazawa Medical University, Uchinada, Ishikawa, Japan
- Janice D Longstreth, Batelle Pacific Northwest Laboratories, Washington, DC, USA
- David H Sliney, US Army Environmental Hygiene Agency, Aberdeen, MD, USA
- Hugh R Taylor, Royal Victorian Eye and Ear Hospital, East Melbourne, Victoria, Australia
- Gijs FJM Vrensen, Netherlands Ophthalmic Research Institute, Amsterdam, The Netherlands
- Alfred R Wegener, Bonn University, Bonn, Germany

Simon P Wolff, University College London Medical School, London, UK, acted as Chairman and Discussion Leader at the workshop. This report is partly based on a 'Chairman's Report' written by him.

Format of the workshop

The format of the two-day workshop was a series of 30-minute talks followed by 15-minute discussion periods. Each half-day session was followed by a period of general discussion.

The first day was primarily devoted to experimental studies, both with cultured lenses and with experimental animals, while the epidemiological studies were discussed on the second day. The workshop closed with a general and synthesising discussion.

Summary of the presentations and discussions

In the discussion of the experimental studies several possible mechanisms were described for the development of opacities in the lens that ultimately may lead to cataract. It is not yet completely clear how these opacities develop. Certain compounds present in the lens are able to absorb UV radiation, and this can be the start of a chain of reactions that lead to the denaturation of lenticular proteins. The most important of these compounds are tryptophan, kynurenines and the chromophores that are

responsible for the yellow discoloration of the lens that occurs in the course of life. According to Dillon, in young lenses approximately 95% of the incident UV radiation is absorbed by kynurenines and 5% by tryptophan. Kynurenines are relatively inefficient at transferring the energy absorbed from UV radiation. During ageing there is a gradual shift to absorption by yellow pigments. These also are not very effective at transferring radiation energy, but they do absorb effectively. Dillon stated that damage seen in older human lenses develops from the accumulation of damage caused by absorption of UV radiation by kynurenines and yellow pigments. Although tryptophan is much more efficient at transferring the energy of UV radiation, the presence of this compound has only been demonstrated in animal, but not in human lenses. According to Hightower, however, it is possible to demonstrate the presence of tryptophan in the epithelium of human lenses. He presented a model in which UV radiation induces changes in the epithelial cells. These changes are passed on to cells in deeper layers by cellular interactions. This could solve one of the problems in the extrapolation of animal data to humans.

Another problem is that animal experiments have been performed almost exclusively with nocturnal or twilight animals. According to Vrensen such animals are a less suitable model for the investigation of the influence of UV radiation on human eyes, since significant differences might exist between these animals and humans in the structure and biochemistry of the lenses and in lifespan. Also animal experiments, in which short intense irradiations are generally given, are not very well comparable to human exposure, that occurs with a low intensity but over long periods.

The general impression from this part of the workshop was, however, that animal experiments and studies on surgically removed human lenses have provided data that support the hypothesis that UV radiation plays a role in the development of cataract. One of the requirements for using epidemiological data to support a cause-effect relation is the existence of a plausible biological mechanism. This requirement seems to have been met in the case of UV radiation and cataract.

Before the presentations on the effects in humans, Sliney gave an overview of intraocular UV dosimetry. He stressed that the eyes are significantly shielded from direct irradiation as a result of their location in the orbits, the presence of the eyebrows, and squinting as a reaction to high light-intensities. There is a significant contribution of both scattered and reflected UV radiation to the total UV burden of the eyes. This is of major importance in the interpretation of the data from epidemiological studies that have not attempted to quantify individual exposure, but instead have estimated UV exposure from local circumstances, such as latitude, elevation and terrain.

Taylor presented an overview of the study on the Maryland Watermen. This epidemiological study is one of the few in which much effort was put into the determination

of individual exposure. However, since it is a retrospective study, it still is a problem that exposure assessment was partly based on the memory of the subjects. Nevertheless it was possible to obtain a fairly accurate estimate of individual exposure. A distinction could even be made between exposure to UV-A, UV-B, blue light and visible light. Another and more important problem with this study is that, rather than the presence of cataract, i.e., visually impairing opacities, the presence of small local opacities was investigated and these can only be found with special equipment. In the Watermen study, opacities on the anterior cortex appeared to be related to the accumulated amount of UV-B radiation and a weak dose-response relation was found. Opacities at other locations in the lens did not show such a relation. No relations at all were found for UV-A, blue and visible light. A separate study in the same region, among patients suffering from posterior subcapsular cataract, also showed a relation between this type of cataract and exposure to UV-B radiation.

Dolin summarised the results of the most important epidemiological studies that had included individual determination of exposure, stratified to the location of the opacities in the lens, i.e., cortical, nuclear and posterior subcapsular. In addition to the results of the Maryland Watermen study, a positive relation between sunlight exposure and cortical opacities was also found in the Beaver Dam study, but only in men and not in women. According to Klein, one of the investigators in this study, a striking observation was the predominance of these opacities in the nasal quadrant of the lens: Sliney had indicated that there are reasons to assume that light entering the lens laterally can be focused precisely in this part of the lens. A study performed in Parma (Italy) showed that outdoor work and leisure activities in sunlight are also related to cortical lesions.

No single study has shown a relation between sunlight exposure and opacities in the nuclear region of the lens.

The patient study in Maryland was the only one that showed a relation between sunlight exposure (or, more specifically, exposure to UV-B) and posterior subcapsular cataract.

Reports of a number of other epidemiological studies are available, but these studies did not include determination of individual exposure or specification of the type of cataract. In these studies, exposure was generally correlated with geographical factors such as latitude and elevation. Several studies also showed a relation between some proxy for sunlight exposure and cataract incidence. Javitt for instance showed that less cataract surgery is performed in the northern part of the USA than in the south.

A major problem with the epidemiological studies is the existence of several factors that can bias the results and the fact that they were not always corrected for adequately. Because of this, Harding argues that a relation between sunlight exposure and cataract has not been demonstrated. Since, however, this was done for other factors

such as diabetes, malnutrition and hygiene, the speaker felt it more useful to focus on these factors. Harding also considered the absence of a relation between cataract and pterygium and pingueculum an important argument against the role of sunlight (or UV radiation) in the development of cataract. There are indications that sunlight is a causal factor for these corneal lesions. Finally, Harding also felt that experimental data provide too few indications of the existence of a plausible biological mechanism for the induction of cataract by UV radiation.

The final outcome of the workshop was the persistence of the lack of consensus between supporters and opponents of the sunlight hypothesis. There was some approach to a consensus regarding posterior subcapsular cataract, the only type of cataract that has been studied directly, and in which lenticular opacities in the subjects were accompanied by decreased visual acuity. The weak relation between sunlight exposure and relatively minor cortical opacities, and the absence of such a relation with cortical cataract *per se*, was no reason for Harding to change his point of view. Other experts felt, however, that such opacities can develop into full-blown cortical cataract and that there is therefore reason to assume that sunlight does have an influence on this type of cataract also.

On the other hand, and all participants agreed about this, it is very likely that if there is an influence of sunlight, it probably is not major compared with that of other factors such as those mentioned by Harding. Sunlight is probably not the most important causal factor in the development of the many cataracts found in countries located in low-latitude regions.

A final point of consensus was the need for more studies such as that with the Maryland Watermen, in which the most accurate possible assessment of individual exposure is made.

Annex **D**

List of abbreviations

- *a*
  experimental period (mouse) or age (man)
- *AIDS*
  acquired immunodeficiency syndrome
- *ALM*
  acrolentiginous melanoma
- $A(\lambda)$
  action spectrum
- *BCC*
  basal cell carcinoma
- Br_2^-
  bromide radical
- *c*
  velocity of light in vacuum
- *C*
  carbon
- Ca^{++}
  calcium ion
- *CBS*
  Central Bureau of Statistics
- *CC*
  cytosine dimer

- *CFC*
  chlorofluorocarbon
- *CI*
  cumulative (tumour) incidence
- *CIE*
  Commission Internationale de l'Éclairage
- *CMM*
  cutaneous malignant melanoma
- CO_2
  carbon dioxide
- CO_3^-
  carbonate radical
- *COS*
  carbonyl sulfide
- *Cu*
  copper
- *DMS*
  dimethyl sulfide
- *DNA*
  deoxyribonucleic acid
- *DOC*
  dissolved organic carbon
- *f*
  frequency
- *FAMMM*
  familial atypical multiple mole melanoma
- *Fe*
  iron
- *H*
  dose
- $H(\lambda)$
  dose at wavelength l
- H_{eff}
  spectrally weighted dose, effective UV dose
- $H_{SCUP\text{-}h}$
  effective dose weighted according to the SCUP-h action spectrum
- *HIV*
  human immunodeficiency virus

- H_2O_2
  hydrogen peroxide
- *I*
  tumour incidence
- *IFN-γ*
  interferon-γ
- *IRRI*
  International Rice Research Institute
- λ
  wavelength
- *LMM*
  lentigo maligna melanoma
- *M. Bowen*
  Morbus Bowen
- *Mn*
  manganese
- *mRNA*
  messenger ribonucleic acid
- NH_4
  ammonia
- *NK cell*
  natural-killer cell
- *NM*
  nodular melanoma
- *NMSC*
  non-melanocytic skin cancer
- NO_x
  nitrogen oxides
- *N-ras*
  a proto-oncogene
- O_2^-
  superoxide radical
- O_3
  ozone
- *OH•*
  hydroxyl radical
- *p53*
  a tumour-suppressor gene

- *PAC*
  polycyclic aromatic carbon
- *PALGA*
  pathological-anatomical national computerised archive (pathologisch-anatomisch landelijk geautomatiseerd archief)
- *RB meter*
  Robertson-Berger meter
- *RNA*
  ribonucleic acid
- *RR*
  relative risk
- *SCC*
  squamous cell carcinoma
- *SCUP*
  skin cancer Utrecht Philadelphia action spectrum
- *SCUP-m*
  mouse SCUP action spectrum
- *SCUP-h*
  human SCUP action spectrum
- *(s)MED*
  (standard) minimal erythema dose
- SO_2
  sulfur dioxide
- *SPF*
  sun protection factor
- *SSM*
  superficial spreading melanoma
- *Th1 cell*
  T-helper-1 cell
- *TNF-α*
  tumour-necrosis factor α
- *TT*
  thymine dimer
- *UV*
  ultraviolet
- *UV-A*
  ultraviolet radiation 315-400 nm
- *UV-B*
  ultraviolet radiation 280-315 nm

- *UV-C*
  ultraviolet radiation 100-280 nm
- *XP*
  Xeroderma pigmentosum
- *VF*
  amplification factor: percent increase in tumour incidence per percent ozone decrease
- VF_b
  biological amplification factor
- VF_o
  optical amplification factor

Units

d	day
h	hour
Hz	Hertz
J	Joule
l	liter
m	meter
M	molar
s	second
W	Watt
DU	Dobson unit

Prefixes

P	Peta: 10^{15}
G	Giga: 10^{9}
M	Mega: 10^{6}
k	kilo: 10^{3}
c	centi: 10^{-2}
m	milli: 10^{-3}
μ	micro: 10^{-6}
n	nano: 10^{-9}