Climate Change Research: Evaluation and Policy Implications
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ASSESSMENT REPORT ON NRP SUBTHEME

"EFFECTS OF INCREASING UV-B RADIATION"

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ABSTRACT

Solar UV-B radiation has profound effects on many organisms. Increases in this type of radiation may, therefore, be expected to have impacts. In this NRP-theme, investigations were carried out on effects of increased UV-B radiation on the immune system, plants from terrestrial ecosystems and algal communities.

These three topics were selected on the basis of expertise available in The Netherlands; the topics chosen ranked high in several international priority listings.

The projects are described, and the results assessed. Each of the projects produced new knowledge on the effects studied. Several results were taken up in the 1994 UNEP-Assessment of Environmental Effects of Ozone Depletion.

Reasons for continuing and intensifying this type of research are given. Other reasonings, implying that such research would not be necessary, are weighed and found wanting. Effects of increasing UV-B radiation rank high among the impacts of global atmospheric change on organisms.

1. INTRODUCTION

1.1 Why research on UV-B effects?

Changes in the atmosphere become relevant to society only if they have effects. This places attention for effects in a crucial position in a research programme on atmospheric change.

The present assessment deals with effects of increased penetration of solar UV-B radiation to the earth's surface, as a consequence of decreasing total-column ozone. UV-B radiation is the most energetic component of the sunlight reaching the surface. Even without change it has profound influences on humans, animals, plants, microorganisms, materials, and on the chemistry of the atmosphere. A possible increase of this type of radiation demands careful attention.

1.2 Selection of projects

Effects of sunlight on organisms are studied in the science called *photobiology*. The Netherlands have a strong tradition in photobiology.
The emphasis has been on human health from the time between the two world wars. Interest in effects on plants and aquatic organisms developed especially in connection with the problem of depletion of the ozone layer.

Selection of topics for NRP-I was guided by the expertise available in the country. This occurred in a spontaneous way, because it was in these areas that project proposals were submitted. This led to the three projects listed in Table 1.1.

Table 1.1
List of projects in NRP Subtheme "Effects of increasing UV-B radiation"

Title	Project leader	Number
Effects of UV-B on the immunological resistance to tumours and infections	H. van Loveren	850017
Impact of enhanced solar UV-B radiation on plants from terrestrial ecosystems	J. Rozema	850022
Effects of increased UV-B radiation on structure and functioning of algal communities in different climatic zones: risk of a global decrease in stratospheric ozone	L. van Liere	851054

The Netherlands provide about 1 percent of the research efforts in the world, and in some areas where it is relatively active a few percent. It was not considered necessary, therefore, that the NRP-projects would provide complete coverage of this worldwide problem. Important areas, such as effects of UV radiation on the eyes, and on materials, were lacking. On the other hand, the topics that were covered belonged without exception to the "key areas of uncertainty" defined by the United Nations Environment Programme, and the priorities defined by SCOPE, the Scientific Committee on Problems of the Environment, formed by the International Council of Scientific Unions.

2. INVESTIGATION OF THE EFFECTS OF UV-B ON THE IMMUNOLOGICAL RESISTANCE TO TUMOURS AND INFECTIONS

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2.1 Introduction

Scientific tools to perform investigations on the health effects of increased UV-B exposure are available. From experimental studies it can be concluded that UV-B causes an increased incidence of tumours by at least two separate mechanisms: 1) by genotoxic activity of UV-B and 2) by affecting the immunologically mediated resistance to tumours. In laboratory animals and also in humans evidence has been provided of UV-B induced damage of the immune system. For this reason it is reasonable to expect that also immunological resistance to infections may be at risk after UV-B-exposure. It is known that resistance against infectious agents that enter the body via the skin, such as Leishmania, and Herpes simplex, is affected by UV-B irradiation. Whether resistance to infectious agents that enter the body via other routes than the skin is affected is as yet not known. Because UV-B can induce systemic immunosuppression, also suppression of immunity against non-skin associated infections (diseases), may be suspected. The main goal of this project was to study/analyze the effects of UV-B on the immune system and resistance to tumours and infections. In these studies dependency of UV-B induced alterations of the immune system on UV-B dose, UV-B wavelength (spectrum) and species (rat, mice, man) were investigated. This information can serve as a basis for estimation of the health risk of UV-B exposed population groups.

2.2 Basic interspecies comparisons

Experiments have been performed in which mice and rats are exposed to UV-B using the newly developed and standerized equipment (FS40 UV-B sunbanks). The MED (minimal erythema, or edema, dose) values were determined for mice and rats. A quantitative scoring method for the determination of UV-B effects on the skin was developed. Using this method it was possible to quantitate edema, redness and swelling reactions after UV-B exposure. In addition to these macroscopical studies also histological studies were performed. Pathological effects such as acanthosis, hyperkeratosis, parakeratosis, and inflammation were induced by UV-B irradiation. These pathological parameters were UV-B dose and time dependent. Similar experiments were performed with a Kromayer lamp as an UV-B source. This lamp was used for local/acute UV-B irradiation. Results obtained with this equipment are compared with results obtained with the FS40 lamps (total body/semi-chronic). Kromayer lamps are widely used in clinics for UV-exposure of humans because it is easily to expose humans with this lamp. Results obtained with the Kromayer lamp in animal species and humans are being compared in order to be better able to extrapolation animal data. Parameters that are being analysed are: acanthosis, parakeratosis, hyperkeratosis, inflammation and macroscopic changes due to UV-B exposure.

Based on these initial studies (characterization of primary skin effects due to UV-B exposure in man and different animal species), the effects of UV-B exposure on several basal specific and non-specific immune parameters were tested. Investigations with respect to changes in the number and subtype of immune competent cells in the skin as well as in lymphoid organs are being carried out. In addition to these descriptive studies also immune function studies are being carried out. Because the mixed lymphocyte reaction (MLR), which is a general parameter for T cell immunity, is often used in studies that deal with UV-B induced changes of the immune system (in rodents and man) we developed the MLR technique at the RIVM. This model is now operable in rats, mice and man. UV-B exposure (in vivo) of mice and rats inhibited the MLR significantly, dependent upon UV-B irradiation-dose and irradiation-time. The MLR will be one of the parameters to be used for the comparison of UV-B effects on T cell immunity between different species (mice, rats, man). For this purpose MLR experiments with human cells were carried out. The effect of in vitro UV-B irradiation of rodent and human blood cells was also studied. UV-B suppressed significantly MLR responsiveness of blood cells in humans and rodents after in vitro UV-B exposure of lymphocytes. Recently we developed (in collaboration with dr. M. Mommaas, AZL), a method in order to investigate the capacity of skin cells to induce MLR responses of allogenic blood lymphocytes. This assay is called the Mixed Skin Lymphocyte Reaction (MSLR). In vivo, in vitro, as well as in situ UV-B exposure of skin cells from different species including man are being carried out. The in vitro irradiation experiments have recently been finished and the comparison of UV-B sensitivity with respect to this immune parameter for the capacity to induce a lymphocytic reaction was analysed, and accepted for publication in "Photochemistry and Photobiology". Histological parameters, obtained in the same species, will be added to these MLR and MSLR results for a better comparison of UV-B sensitivity/susceptibility, between the different species. Other immune function tests such as natural killer cell function and mitogenic responses to several different mitogens were also significantly altered in rats after in vivo UV-B exposure. Mice and human studies are planned with respect to these parameters. In vitro UV-B exposure of lymphocytes from rodents and man inhibited mitogenic responses significantly.

2.3 Infection models in rodents

The effects of UV-B exposure on (immunological) resistance against infectious diseases (bacteria,parasites and viruses) is studied in rats and mice. For this goal several infection models in rodents are available at the RIVM. At this moment a Listeria model (bacteria) and a Trichinella model (parasite) and a Cytomegalo virus infection model are operational. In collaboration with the EPA (Selgrade; Environmental Protection Agency, Health Effects Research Laboratory, section of Immunotoxicology, Research Triangle Parc, NC, USA) an additional virus model is used. Using this collaborative study the effect of UV-B exposure on the immunological resistance to Influenza infections in the rat will be investigated. Recently experiments were performed in order to test the effect of UV-B on the resistance to malaria infections in the rat. This part of the resistance study is done in collaboration with Dr. Luebke from EPA, Research Triangle Park, NC, USA. All infection models that are used are in principle suitable to test immunosuppression, and hence are also suitable to test possible immunosuppression induced by UV-B. If all the available infection models are used almost all the different aspects of immunological resistance against infective agents are investigated.

We demonstrated that UV-B exposure (suberythemal doses) can inhibit the immunological resistance against Trichinella spiralis infections in rat. Especially UV-B exposure during the second week after Trichinella infection inhibits resistance against this pathogen indicating that T cell dependent immune responses are probably altered. In addition, specific IgE titers in serum of UV-B exposed animals are decreased if the animals were irradiated 3 weeks after Trichinella infection Additionally, we demonstrated that suberythemal doses of UV-B inhibited the resistance against a Listeria monocytogenes infection. Especially the specific T cell response to this pathogen was inhibited by UV-B exposure. A manuscript describing these results is now accepted for publication in "Environmental Health Perspectives". As was found for the parasitic and bacterial infection models also resistance to cytomegalovirus was inhibited by UV-B exposure of rats.

Therefore, the important new finding is that resistance against several non-skin-associated infectious diseases can be inhibited by UV-B exposure.

2.4 Immunological changes in relation to UV-induced skin cancer

Extensive experimental studies on UV-induced skin carcinomas have been carried out with albino hairless mice (SKH:HR1) in order to extract basic quantitative dose-response relationships for modelling of human skin cancer risk. From other animal models it was known that UV tumours are highly antigenic, i.e., these tumours are rejected by a strong immune reaction when transplanted into a genetically identical animal. Somehow the UV-exposed animals loose this ability. In the present project lymphoid organs and skin of the hairless mice were investigated in the course of chronic UV-B exposures, leading up to skin carcinomas. Important shifts in subpopulations of lymphoid cells were found very early in the experiment in the spleens and especially in the lymph nodes of the animals. CD8-positive cells migrated into the superficial layers of skin in the course of the experiment. By challenging the mice after different times and levels of UV exposure it was ascertained how the observed shifts in subpopulations of lymphoid cells correlated to the failure to reject UV tumour cells. This failure sets in long before the occurrence of macroscopically observable, UV-induced primary tumours, it gradually develops after the initial shifts in subpopulations of lymphoid cells. The relatively rapid suppression of the immunity against the UV carcinomas may suggest that this is not a serious rate-limiting step in the genesis of these cancers (and thus difficult to prevent in high risk individuals). Papers on this subject are in preparation and will be submitted this year. Early detection of corresponding immunological changes in humans could serve to identify individuals that run a high risk for UV-related skin cancers and perhaps also for decreased immunity against certain infections.

2.5 Basic mechanisms

Besides the aforementioned interspecies comparisons of UV sensitivities, a basic understanding of the mechanisms involved in UV-induced immunosuppression is likely to further a well-directed research on UV-related immunological risks in man. Although fundamental research on mechanisms is carried out by several groups, this research has not yet yielded data that provide good answers on how the results obtained in animals translate to humans. Hence, experiments were carried out to contribute to this animal-to-human extrapolation. We did this by exploring the in vivo murine model for fundamental immune parameters that would directly signify a high sensitivity to UV-suppression, or a state of UV-induced immune suppression, and that would also be measurable in man.

To capitalize on a well-established assay at the RIVM on the delayed type of hypersensitivity (dth), we studied the effect of UV irradiation on the sensitization for the early (initiation) phase of the hypersensitivity reaction against picrylchloride applied to the skin. Besides the already-known suppression of the classical dth reaction (swelling at 24 h after the application), we found a suppression by UV radiation of the early phase of dth (swelling at 2 h). Both phases in the dth reaction are mediated through T cells, and we found - contrary to our expectation - that suppression of the late phase did not fully correspond with the suppression of the early phase (the early phase could be made to occur in conjunction with an UV-suppressed late phase dth reaction; see Y. Suntag et al., 1994). An antigen-specific factor (in this case picrylchloride-specific) 'arms' mast cells in the skin for the induction of increased permeability of bloodvessels, causing the edema of the early phase dth reaction. Such a factor should in principle also be measurable in humans, and might be used to measure a person's UV suppressibility.

A very interesting finding stemmed from the combination of the present research on UV immunological effects with a parallel running research project on DNA damage induced by UV radiation. UV-specific DNA damage can be detected through the binding of a monoclonal antibody (H3, supplied by Dr. L. Roza at TNO in Rijswijk) to UV-induced thymine dimers in the DNA. Upon UV irradiation the DNA damage is normally confined to the most superficial layers of skin (the damaging UV radiation does not penetrate any deeper), but UV-damaged DNA was also detected in some cells the draining lymph nodes, already 1 hour after UV exposure. By a cumbersome combination of techniques, it was ultimately established that these UV-damaged cells from the skin were in fact antigen-presenting cells (APCs). UV-damaged APCs are known to be (partially) dysfunctional, which can result in unbalanced, aberrant immune reactions (no stimulation of Th1 cells, but only of Th2 cells exerting suppressive action). It is now of great importance to establish whether UV-damaged APCs (more particularly, Langerhans cells) migrate from the human skin upon UV irradiation. Such a follow up study is currently pursued with a high priority. Enhanced migration of UV-damaged APCs in some individuals (e.g. people who had skin cancers removed) could signify an increased risk for UV-induced immune suppression.

2.6 Conclusions

The present NRP-I project has been extremely rewarding in that it produced a good many new data on which to base an animal-to-man extrapolation of UV-induced suppression of immune reactions, such as involved in combating infections and skin cancers. Animal experiments widened the spectrum of infections that could be adversely affected by UV radiation, by including infections not entering through the skin. Thus, the possible impact of an increase in UV-B radiation has been importantly broadened.

The well-directed study has identified some promising immunological parameters by which to establish an individual's UV-immunosuppressibility, or a state of immunosuppression against certain (infectious) agents. Further studies are needed to establish the feasibility of measuring these parameters in humans for the proposed diagnostic purposes. Clearly, this research has not yet fully evolved to the point that quantitative assessments of UV immunosuppression, and especially the repercussions on infections and vaccinations can be reliably made. The mathematical centre of the RIVM is, however, carrying out preliminary studies on developing a suitable risk model based on the data generated in the present project. In conjunction with this study, the centre of epidemiology at the RIVM is exploring the possibilities of providing epidemiological data (e.g. influenza data in relation to sun hours during summer) to support assessments of UV effects on infections or vaccinations. As such epidemiological data may not be available in a suitable form, plans are also being made for dedicated surveys in which people will be interviewed to investigate a possible relationship between (solar) UV exposure (during vacations) and subsequent risk or severity of infectious diseases.

Although it is not part of our daily experience, animal experiments show that UV radiation from the sun may interfere with our immunity against infections. The relevancy of this UV-immunosuppression for infections and vaccinations in a human population still remains to be established: it is unknown whether we are all indiscriminantely at risk of UV-immunosuppression, or whether the risk pertains solely to high risk individuals (e.g. people already immunocompromised in some way), or whether the risk is nonexistent. Judging by the available experimental data, also supplied by the present project, the latter alternative does not appear to be the most plausible one. Hence, a continuation of this line of research is well warranted, because our ignorance on the subject hampers a good appreciation of health effects of UV radiation, and of increases in UV radiation due to an depletion of the ozone layer.

2.7 Scientific cooperation

In this NRP project the research groups of Dr. F. de Gruijl (RUU, laboratory for dermatology) and of Dr. H van Loveren (RIVM, laboratory for pathology, section of immunobiology) have been and still are collaborating intensively. Knowledge with respect to Ultraviolet radiation and the immune system are connected due to this collaboration.

In addition to this formal collaboration five times a year a scientific meetings among researchers in The Netherlands, concerned with UV-B effects on the immune system are organized. During these meetings plans/results from the participants with respect to research concerning UV-B induced alterations of the immune system are discussed. The participants of this national UV-B network are:

1. RUU, Laboratory for Dermatology, Utrecht.
2. RIVM, Laboratory for Pathology, section Immunobiology.
3. LU, Laboratory for Dermatology, Leiden.
4. UvA (AMC), Laboratory for Dermatology, Amsterdam.

In addition to these meetings regarding health effects of UV-B two times a year a meeting is arranged in which the other UV effects projects, financed by NRP, participate (Free University, Amsterdam; RIVM, Bilthoven; State University, Groningen; State University, Utrecht). The first of these meetings was in November last year at the Free University in Amsterdam. The second UV-B-effects cluster meeting was at the RIVM in April 1993. This meeting was very successful with respect to the integration of the different UV-B "effects studies". The third and fourth meeting were held in Utrecht respectively Groningen (November 1993 and june 1994).

In addition to the national collaborations as mentioned above, the RIVM is coordinating a project sponsored by the EC, entitled: "*A basis for better evaluation of risk of increasing UV-B exposure for public health*". For this purpose collaboration with several groups in Europe is established: Dr. Dall'acqua, Padua, Italy; Dr. Cerimele, Rome, Italy; Dr. Norval, Edinburgh, Scotland; Dr. Gibbs, Dundee, U.K.; Dr. de Gruijl, Utrecht, The Netherlands. The knowledge/expertises of these different labs will serve to further evaluate the risk of increasing UV-B exposure on public health. The accessibility to human data with respect to UV-B induced effects obtained by several of the participants is now possible.

2.8 Effects of UV-B on the immunological resistance to tumours and infections

Assessment
It was known from experimental work by other groups that UV radiation could influence the course of certain infections. That were infections where the skin was involved, either as the site where the infection entered the body, such as in Leishmania, or where the infection came to expression, such as in Herpes simplex. In the present project it was shown that UV-B irradiation of the skin of experimental animals can also aggravate the course of an infection that has nothing to do with the skin, for instance, a worm infection that enters the body with the food and has its damaging effects deep inside the body. This surprising finding broadens the range of infectious diseases that require attention as diseases possibly to be influenced by increasing solar UV-B radiation. The result is being included in the UNEP Assessment on Environmental Effects of Ozone Depletion (1994), as has been the case with several experimental results from this group in the earlier UNEP assessments.

Another valuable result obtained in this project was the finding of cells with UV-B induced damage to their DNA in the deep lymph nodes of mice. Because of the limited penetration of UV-B radiation in animal tissues, this damage must have been inflicted while the cells were in the skin. This finding confirms an important part of the theoretical model on how the immune system is influenced by UV radiation, and strengthens the basis for the prediction of the ultimate effects.

3. IMPACT OF ENHANCED SOLAR UV-B RADIATION ON PLANTS FROM TERRESTRIAL ECOSYSTEMS

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3.1 Introduction

Background
Decrease in stratospheric ozone over the last decade has been revealed by satellite measurements. As a result a gradual increase in solar ultraviolet-B radiation occurs. Measurements of UV-B radiation in the Swiss Alps indicate an annual increase of UV-B-radiation of 1% since 1981 (Blumthaler and Ambach, 1990).

Solar UV-B and terrestrial ecosystems
Solar UV-B radiation has important biological and ecological effects. Many crops and natural plant species demonstrate reduced growth, under enhanced UV-B

radiation but there is a wide range of sensitivity to UV-B radiation between plant species and cultivars. A major part of research of UV-B effects relates to crop species and only a few UV-B effect studies are known of plant species of natural ecosystems (SCOPE 1992).
Most studies of UV-B effects on plants have been conducted in greenhouse or in controlled environment cabinets with UV-B lamps. Relatively low levels of Photosynthetic Active Radiation (PAR), in controlled environment studies may prevent induction of photo-repair of UV-damage. This has led to an overestimation of growth reduction of plants exposed to enhanced levels of UV-B. Outdoor experimental UV-B supplementation systems, with natural levels of PAR provide more realistic responses of plants to enhanced UV-B.

Scientific aims
Our research aimed at development and application of outdoor UV-B supplementation and UV-B filtrations systems and to assess effects of solar UV-B radiation to plant species of terrestrial ecosystems.

3.2 Methodology

UV-lamp systems
In experimental studies with enhanced levels of UV-B irradiance UV-lamps are used that are pre-burnt and filtered with cellulose acetate as a cut off filter of wavelengths smaller than 280 nm. The Cellulose Acetate filters are renewed twice a week to avoid reduced transmission of UV-B related to ageing of the cellulose acetate. Mylar polyester filters absorb UV-B and are used to cover UV-B lamps over control treatment that receive no UV-B irradiance, but where the UV-B levels are the same as in the treatment that receive no with enhanced UV-B (see Figure 3.1).

Figure 3.1
Philips TL12/40 lamps are applied both in indoor and outdoor studies of UV-B effects on plants. The plots shown in the photograph, which are exposed to solar UV-B + UV-B supplied by the lamps, refer to monocultures and mixed cultures of the dune grassland species *Calamagrostis epigejos* and *Holcus lanatus*. Thus the effect of enhanced UV-B on competitive relationships between plant species is analysed

We use Philips TL12/40 lamps both in indoor controlled environment studies and in outdoor studies in an experimental field and in natural ecosystems.
Two outdoor unenclosed systems are applied:
A. UV-B Supplementation system and
B. UV-B filtration systems.

UV-B Supplementation system. A lamp system to supplement ambient solar UV-B over experimental plots and over natural vegetation has been developed and applied (Figure 3.1).
At present this outdoor UV-B supplementation system is applied in a "square wave" mode of enhanced UV-B radiation. This implies switching UV-B lamps on for a fixed period around midday, when natural solar UV-B is greatest.
Currently an outdoor solar tracking UV-B supplementation is developed and installed. This involves continuous measurement of solar UV-B levels using appropriate UV-B sensors (Yu et al. 1991). This allows accurate simulation of enhanced UV-B throughout the day. There is evidence that plant responses to enhanced UV-B supplied in the solar tracking modulated mode differs both qualitatively and quantitatively from responses to UV-B supplied in the square wave mode.
The development and testing of this solar tracking UV-B supplementation is in close cooperation with Dr. Andy McLeod, Institute of Terrestrial Ecology, Huntingdon, United Kingdom and Dr. Gaetano Zipoli, IATA-CNR, Florence, Italy.
UV-B lamps are mounted above the vegetation and are maintained at a constant height above the canopy. UV-B sensors will measure and track ambient solar UV-B radiation. Dual UV-B sensors are used beneath both treatment and control racks with UV-lamps, spaced such that one is unshaded from direct sunlight by lamps or frame. The unshaded sensor is selected by the control system preventing shading effects from interfering with feedback control of output of UV-lamps. The lamp output is adjusted to give a constant multiple of UV-B radiation above ambient UV-B radiation. Increases of UV-B radiation relative to ambient UV-B irradiance relating to 20% ozone depletion under clear sky are realized.

UV-filtration system. Various types of solid plastic filters are installed above precultured plants in pots or above natural vegetation. These filters absorb either (a) little solar UV-B (and UV-A) (Acrylate filter), (b) all UV-B (Mylar polyester film on top of acrylate filters) and (c) all UV-A + UV-B (Lexan filter).

The transmission spectra of these three filter types are given in Figure 3.2.

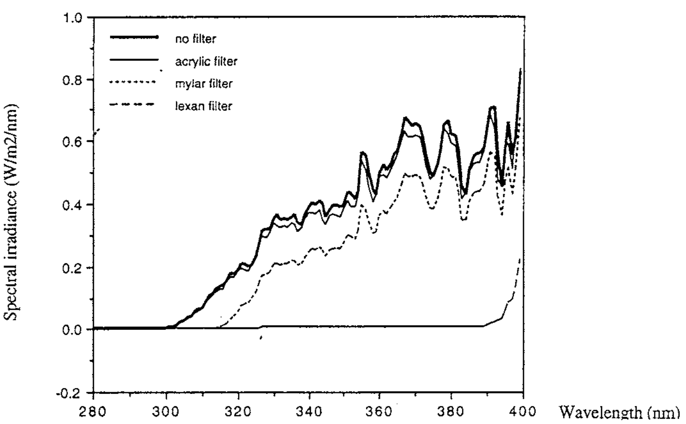

Figure 3.2
Solar UV-irradiance (August 1992, 13.00 h), on a clear sky day, beneath the various filter types (A = acrylate, L = Lexan, M = Mylar), compared to full sunlight (no filter). Measurement with a Optronic spectroradiometer (OL 752, Optronics Laboratories, Florida, USA)

The development and testing of low-cost solid plastic UV-B filters allow outdoor and ecosystem studies of reduction of ambient levels of solar UV-B radiation. In practice it will allow research of responses of plant from natural terrestrial ecosystems (Figure 3.3).

3.3 Responses of terrestrial plants to enhanced solar UV-B irradiation

Solar UV-B effect studies have been performed on a number of crop and native plant species both in controlled environment (greenhouse), unenclosed outdoor (experimental garden) and field studies in a dune grassland ecosystem (Tables 3.1 and 3.2).
A wide range of sensitivity to enhanced UV-B measured in indoor controlled environment experiments, exists among crop species. Cultivars of crops such as soybean (*Glycine max*) vary greatly in sensitivity to UV-B.

Of the natural plant species from Dutch coastal ecosystems: the salt marsh ecosystem and dune grassland ecosystem, no apparent sensitive plant species were found both in indoor and outdoor studies. It should be noticed that no natural plant species of the Leguminosae (a plant group with many plant species sensitive to UV-B) have been subjected to enhanced UV-B radiation levels in outdoor experiments.
The woodland understorey species *Alliaria petiolata* indicates to be sensitive to enhanced UV-B.
It may be that plant species occurring in open habitats (*Calamagrostis epigejos*, *Spartina anglica*) and being exposed to relatively high levels of ambient solar UV-B have evolved UV-B adaptation mechanisms. Other species occurring in the woodland understorey with reduced PAR and reduced ambient UV-B, seem to be relatively UV-B sensitive. This hypothesis is studied in current research.

Figure 3.3
Application of solid plastic UV-B filters in the study of reduction of ambient solar UV-B radiation on plant species of a dune grassland ecosystem. The height of the plastic UV-B filters can be adjusted according to seasonal development of the vegetation. At the background a rack of UV-lamps over the dune grassland vegetation is shown as a UV-B supplementation system

3.4 Ecological effects of ozone depletion; implications for environmental policy

1. Responses of terrestrial plant species to enhanced UV-B range from very sensitive to tolerant or even a positive response. This broad range type of response possibly reflects the intrinsic heterogeneity of the various terrestric ecosystems. Grassland ecosystems are much more exposed to full solar UV-B than under storey plants from forest ecosystems.

2. Adaptation to enhanced UV-B may consist of two types of mechanisms.
   a. UV-B tolerant plant species avoid enhanced UV-B radiation flux at sensitive metabolic sites by absorption of UV-B by epidermal structures and dissolved, UV-B absorbing compounds such as flavonoids.
   b. UV-B tolerant plant species have effective UV-B repair mechanisms, in contrast with UV-B sensitive plant species.
   The nature and occurrence of these adaptation mechanisms has been analysed only for a few plant species. Understanding of UV-B responses of all groups of terrestrial plant species needs research priority.

3. Crop plant species tend to be more sensitive to enhanced UV-B than wild plant species. This may indicate that during the breeding procedure domesticating wild species to crop plants adaptation to (high) UV-B has been lost. Maybe a trade off exists between high productivity and sensitivity to UV-B. This is an intriguing hypothesis. It might indicate that plant species may loose (genetically determined) adaptations to solar UV-B.
   This implies that certain crops or certain cultivars of crops will be less productive with increasing solar UV-B. It is unknown whether selection for

increased tolerance to enhanced UV-B will be successful and over what time period.

4. There are many uncertainties in the knowledge of UV-B effects on plant life in terrestrial ecosystems:
   - effects of UV-B on reproductive biology are largely unknown, some of the reproductive features will be vulnerable to enhanced UV-B;
   - little is known of UV-B effects on "lower plants" like mosses ferns, fungi and algae;
   - the effect of an enhanced UV-B flux is unknown in a "multiple stress" environment such as UV-B air pollution; UV-B x nutrient and water deficiency.

   There are some indications that enhanced UV-B will cause increased occurrence of plant diseases by fungi, bacteria and viruses.

5. Consequences of enhanced solar UV-B to terrestrial ecosystems are expected to be substantial. There will be a shift to dominace of UV-B resistant plant species. The altered biochemistry of plant species exposed to high UV-B fluxes may not only affect plant-herbivore relationships but also change the process of decomposition of leaf litter (see Figure 3.4).

3.5. National and international cooperation

National and International contacts. Between the different national UV-B research groups exchange of experimental results and methods occurs. Every six months a meeting is organized by one of the participating groups. During these meetings results are presented and discussed. Close collaboration between different groups exists on assessing the impact of UV-B on DNA Damage. Immunological techniques used in skin cancer research are now also applied to assess DNA damage in marine algae and terrestrial plants.

Contacts exist with research groups in Germany (prof. M. Tevini, Karlsruhe) and the United States (prof. A.H. Teramura and Dr. J. Sullivan, Univ. of Maryland, Washington), who are well known for their work on UV-B effects on terrestrial plants. Results were presented and discussed during visits to these research groups in 1992, 1993, 1994.

National cooperation

1. Dr. L. van Liere, Dr. A. Veen, RIVM, UV-B and aquatic Ecosystems.
2. Dr. W. Gieskes, Dr. A. Buma, RUG, DNA-UV-B-damage, UV-B dosimetrie.

International cooperation

3. Dr. A. McLeod, ITE, Huntingdon, U.K., UV-B experimental set-up.
4. Prof.Dr. M. Tevini, Dr. J. Ros, TU, Karlsruhe, UV-B spectroradiometrie.
5. Prof.Dr. A.H. Teramura, Un. Maryland & Hawaii,

Dr. J. Sullivan, USA, advanced cooperation and interchange.

International workshop UV-B and Biosphere

The groups involved in this project and in the project on UV-B and algal communities (nr. 851054) will organize an international workshop on the influences of UV-B radiation on terrestrial and aquatic ecosystems. That will be held towards the end of 1995.

Sponsors: the Dutch Ministry of Housing, Physical Planning and the Environment, The Dutch Organisation for Scientific Research, the Royal dutch Academy of Sciences and the European Community.

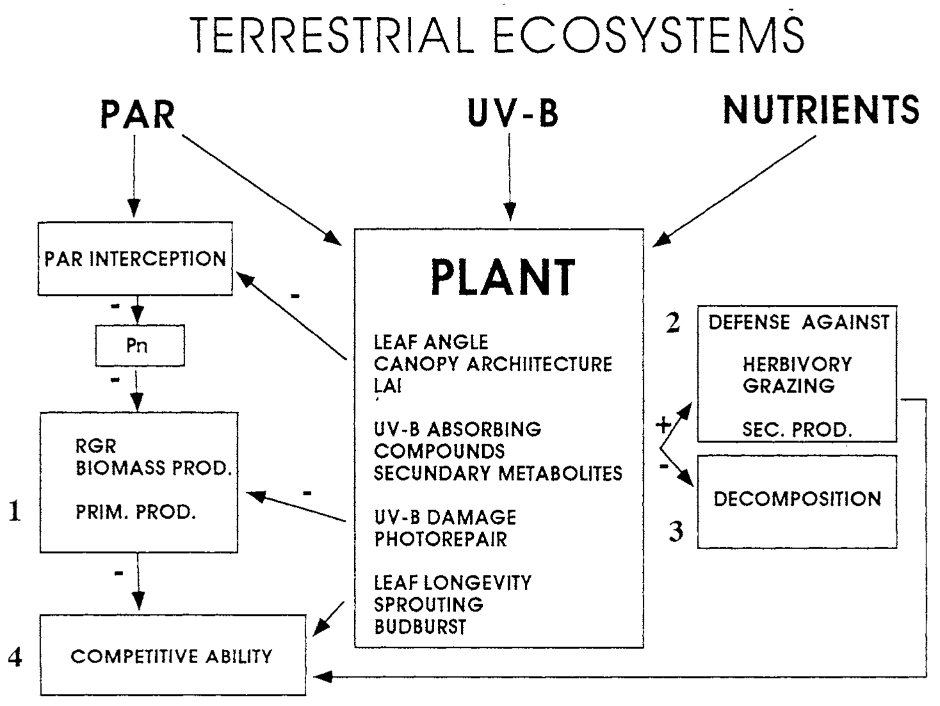

Figure 3.4
Conceptual model structure Impact of Enhanced Solar UV-B on Terrestrial Ecosystems: Physiology, Functioning and Dynamics.
Ecosystem Functions: 1. Prim. Prod. = Primary productivity; 2. Sec. Prod. = Secondary Productivity; 3. Decomposition. Ecosystem structure: 4. Competitive ability.
PAR = Photosynthetic Active Radiation; Pn = Net Photosynthesis; RGR = Relative Growth Rate; LAI = Leaf Earea Index

Table 3.1.
Survey of qualitative plant responses to enhanced UV-B in controlled environment studies. Daily biologically effective dose of UV-B relating to 20-50% ozone depletion under clear sky.

	Response to enhanced UV-B			
	negative very sensitive	response sensitive	no significant response	positive
Crop species				
Pisum sativum	X			
Phaseolus vulgaris		X		
Vicia faba	X			
Lycopersicon esculentum		X		
Cucumis sativus	X			
Triticum aestivum		X		
Zea mays			X	
Natural plant species				
Verbascum thapsus			X	
Calamagrostis epigejos				X
Plantago lanceolata		X		
Alliaria petiolata		X		
Aster tripolium			X	
Elymus athericus		X		
Spartina anglica			X	
Holcus lanatus			X	
Silene vulgaris			X	

The reader is referred to Rozema (1993) for more detailed information

Table 3.2
Survey of qualitative plant responses to enhanced UV-B in controlled environment studies. Daily biologically effective dose of UV-B relating to 20-50% ozone depletion under clear sky

	Response to enhanced UV-B			
	negative very sensitive	response sensitive	no significant response	positive
Crop species				
Vicia faba	X			
Triticum aestivum	X			
Zea mays		X		
Natural plant species				
Calamagrostis epigejos				X
Plantago lanceolata	X			
Urtica dioica		X		
Holcus lanatus			X	
Verbascum thapsus		X		
Silene vulgaris			X	

The reader is referred to Rozema (1993) for more detailed information.

3.6 Impact of enhanced solar UV-B irradiation on plants from terrestrial ecosystems

Assessment
The project gives valuable additions to the international efforts in this field: the inclusion of plants from natural ecosystems, and the investigation of mixed cultures of these plants. These are necessary steps towards the difficult analysis of the influence of increased UV-B irradiance on natural terrestrial ecosystems, a priority topic in several international listings.

The researchers in this project are making progress in identifying those ecosystems, or sub-ecosystems, for which the UV-B irradiance is an important factor. Cultured crops tend to be more sensitive to enhanced UV-B radiation than wild plants, and plants occurring in woodland understoreys more sensitive than plants occurring in open habitats.

4. EFFECTS OF INCREASED UV-B RADIATION ON STRUCTURE AND FUNCTIONING OF ALGAL COMMUNITIES IN DIFFERENT CLIMATIC ZONES: RISKS OF A GLOBAL DECREASE IN STRATOSPHERIC ONZONE

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4.1 Summary

During NRP-I new insights were gained with respect to UV penetration in the aquatic environment and UV stress phenomena in microalgae after chronic exposure to realistic levels of UV-B. UV-B radiation and penetration modelling has revealed that UV-B penetration can be high in natural waters, particularly in the open ocean where the concentration of humic acids is low. Here, the UV-B attenuation coefficient approaches that of pure water. Large differences in UV-B sensitivity were found when microalgae were subjected to chronic UV-B exposure. Especially the growth of species occurring in offshore waters, such as the important bloom forming alga Emiliania huxleyi was already inhibited at ambient UV-B levels: for *Emiliania huxleyi* growth inhibition was found to occur at least up to a depth of 15 m, as calculated for 52°N in the Atlantic Ocean. This indicates that UV-B is a natural selective factor in plankton assemblages and that an increase in incident UV-B levels will strongly affect the performance of many organisms in the field. Furthermore, thanks to our approach of measuring chronic UV-B effects over several generations, we can now present convincing evidence that UV-B effects are underestimated when only short term effects are taken into account, as done so far in UV-B effect studies. This means that the observed 12% decrease in phytoplankton production in Antarctic waters, that is used often for risk evaluation of ozone depletion, strongly needs to be reevaluated, because this study was based on short term photosynthesis measurements. Moreover, growth rate reduction was found to be a more reliable and realistic parameter to be considered than the production measurements done so far, because growth is a resultant of all metabolic processes under UV-B stress, including DNA damage, i.e. not only the photosynthetic process. Therefore chronic UV-B mediated growth inhibition phenomena urgently need to be included in future UV-B effect studies and modelling.
The results obtained during NRP-I have indicated that DNA damage and its subsequent effect on the cell cycle play a major role in UV-B mediated growth rate reduction. This approach is new in aquatic UV-B effect studies and further underlines the importance of studying UV-B effects over several generation times. DNA damage occurs at realistic UV-B levels in most species tested so far, and the ability to overcome DNA damage is now thought to be a primary factor causing difference in UV-B sensitivity in microalgae. The UV-B induced cell cycle arrest, that we observed, causes shifts in mean cell volume and the contents of structural compounds (protein) and storage products. This will definitely result in changes in the food web through altered grazing activities by herbivores with specific food size

requirements, as well as altered sinking rates, resulting in a proportional increase in vertical carbon fluxes out of the euphotic zone.

The experimental results have provided basic insight into the key parameters to be included in effect models, that are designed for risk assessment in specific marine habitats. First modelling on large scale can be realised within the coming years if funding is obtained through NRP-II. The simulation model that we developed recently is focussed on the vulnerability of the Atlantic Ocean ecosystem for integrated climate change factors, including UV-B, temperature and carbon dioxide, and is so far unique within the international scientific community.

4.2 Introduction and problem definition

At present no conclusive picture exists on the impact of enhanced UV-B radiation on the structure and functioning of aquatic ecosystems. The limited knowledge on UV-B leaves enough room for speculation on dramatic effects but also trivialization of the problem. During the past few years evidence for both can easily be found in the media and official reports. It is naive to consider the UV-B problem a passed station following the Copenhagen amendments, first of all because observed ozone depletion levels do not always fit the predicted ozone scenarios and secondly because the effects on ecosystems are still poorly quantified. Therefore, a scientifically sound picture of the effects of UV-B enhancement on ecosystems is required.

In aquatic ecosystems microalgae form the first trophic level. Since microalgae are known to be sensitive to UV radiation, and since UV penetrates to ecologically significant depths in aquatic environments, effects on global primary production upon ozone depletion are likely to occur. Moreover, UV induced changes in primary production will affect the functioning and structure of aquatic food webs.

Laboratory experiments have shown that UV-B affects almost all metabolic processes. This is not surprising: microalgae are unicellular, so the complete organism is subjected to UV radiation. However, as laboratory light conditions usually deviate from the underwater light environment with respect to applied radiation levels and spectral conditions, extrapolation of laboratory findings to the actual field situation is extremely difficult. Therefore, it is crucial for effect studies to assess first of all the primary targets which determine the net result in situ, i.e. the changes in primary production. Available experimental data refer almost exclusively to short term effects. Natural phytoplankton assemblages or species isolated for laboratory experiments are generally exposed to UV-B radiation for only a few hours, while mechanisms operating on a time scale of more than several hours, such as repair of DNA damage and adaptation are not accounted for in such experiments. Nevertheless, these mechanisms may well determine the sensitivity of an organism to chronic enhancement of UV-B radiation. Therefore, experimental data reported so far were not adequate to support a realistic risk analysis. Obtaining such risk analysis was the ultimate goal of our research effort.

Collection of experimental data for the purpose of assessing the quantitative impact of UV-B radiation on natural microalgal assemblages is obstructed by several other problems. UV-B effect studies are complicated by spatial and temporal variations in damaging radiation as well as its interaction with UV-A and visible light, as occurring in the field. Therefore, natural daily courses of UV-B radiation are generally significantly different from experimental exposure regimes. Because several UV induced biological effects appear to be nonreciprocal between dose rate and exposure time, an accurate dosimetry is essential for mimicking natural UV conditions. Furthermore, the spectral distribution of solar light differs significantly from that of most artificial light sources. Since biological effects are known to be strongly dependent on wavelength, biological weighting functions are required for the conversion of radiation spectra to biological effective radiation. So far, no specific "microalgae" action spectrum had been determined for the inhibition of chronic UV-B radiation on algal production. Finally, only few data are available on the penetration of UV (especially UV-B) in seas and oceans under different hydrographical and climatological conditions. Spectral data are needed for the estimation of attenuation coefficients in the ultraviolet range, the assessment of biological effective dose rates at different water depths in the euphotic zone and a realistic assessment of in situ UV responses. This type of data should also form basic knowledge to be used in the establishment of an appropriate dosimetry in laboratory experiments.

Summarizing, at the start of the project no quantitative data were available to assess the effects on aquatic ecosystems. Additionally, the high accuracy necessary for the quantification of UV-B effects made traditional culture methods only poorly applicable. So the generation of accurate and relevant quantitative data required both adequate infrastructure and method development.

4.3 Aim of the project

Considering the above mentioned uncertainties, this project was directed towards the study of long-term effects (several generation times) of enhanced UV-B radiation on algal production. To this end knowledge on the most relevant UV-sensitive metabolic processes had to be gained. This information would then be essential for selecting the most appropriate biological weighting functions (action spectra) for translation of experimental results to field conditions. Furthermore, dose effect relationships had to be established for different species. Also, field measurements of the underwater UV-B light regime were needed to gain knowledge of the vertical extinction of UV-B in various natural waters. The results of both experimental and field measurements would supply basic information to be incorporated in exposure models to assess the effects of various scenarios of UV-B irradiance on specific habitats.

Two main lines of research were pursued:

A. Analysis of in situ UV-B light regimes of different water types (both freshwater and marine). This included: development of a radiation model to calculate surface UV-B irradiation and daily doses; verification of the radiation model by field measurements and by measurements performed by the Laboratory of Radiation Research of the RIVM; field measurements of solar irradiation and spectral attenuation coefficients using an Optronic OL 752 spectroradiometer equipped with a submersible enclosure; measurement of intrinsic optical properties (absorption, scattering coefficients) of suspended material (algae and detritus) and dissolved organic matter for estimation of underwater transmission of UV-B radiation, development of a penetration model to estimate UV-B exposure of algae in different water types and to determine potentially sensitive water types.

B. Experimental studies of sensitivity and protection to natural ranges of UV-B radiation in natural phytoplankton assemblages and unialgal cultures. This included: assessment of dose-response relationships for representative marine and freshwater algae; analysis of reciprocity between dose rate and exposure time; experimental studies on causal relationships between algal sensitivity and relevant physiological mechanisms; development of a computer controlled dynamic light system (DLCS) to simulate natural light regimes in laboratory cultures; development of a special culture system for the assessment of action spectra and analysis of physiological reactions; assessment of a "general" action spectrum for the long-term effect of UV-B radiation on algal growth; integration of effect and exposure data into a general risk assessment of UV-B effects on algal production.

4.4 Results

Infrastructure and methodology built up during the project

- A spectrophotoradiometer (Optronics OL 752), purchased at the beginning of the project, was equipped with a submersible enclosure to allow for spectral underwater measurements to a depth of 12 meters. Expertise was accumulated using this equipment during several cruises (Dutch estuarine waters, open Atlantic waters). The spectroradiometer measurements also provided the possibility to establish an accurate dosimetry in experimental systems.
- Two specialised culturing facilities were developed and made operative: a computer controlled dynamic light system (see below) and a culturing device for measuring wavelength dependent biological effects (action spectra, see below). Special temperature controlled culture rooms were made operative for UV experiments.
- Detailed information was collected on changes in transmission of materials (perspex, cellulose acetate, polystyrene, glass filters) to be used in UV experiments, as a prerequisite for the establishment of an accurate dosimetry during long-term experiments.
- A method was developed to study in vivo DNA damage in individual phytoplankton cells, using immunofluorescent labelling. This has resulted in long-term cooperation projects with other research groups (see 4.7).
- An Image Analysis System was purchased to study DNA damage in natural phytoplankton assemblages. This system was also made operative for accurate cell size measurements.
- A Coulter Counter Multisizer system was purchased eventually to study in detail the UV-B induced changes in cell size and volume.

Radiation and penetration models

A UV radiation model has been developed to estimate natural light regimes as a function of meteorological conditions, season and latitude. Verification of the radiation model was done using measurements performed by the Laboratory of Radiation Research of the RIVM as well as field data obtained with the Optronics OL 752 during several cruises. To allow analysis of under water UV exposure a separate model was developed to calculate the transmission of UV under water. This model relates spectral transmission with intrinsic optical properties: absorption and scattering characteristics of suspended and dissolved substances in various water types. Laboratory and field data on absorption properties of algal cells, detritus and humic acids were collected and included in the model. Through application of the model mean UV exposure levels could be calculated for Dutch aquatic systems. It was found that dissolved organic matter (humic acids) causes most of the attenuation in freshwater and coastal regions. Here the UV-B attenuation coefficient is up to 20 times higher than the attenuation coefficient for visible light. Due to the very low levels of humic acids the attenuation of UV-B radiation in the ocean is relatively low. In clear ocean waters the UV-B attenuation coefficient approaches the theoretical value for pure water i.e. two times the attenuation of visible light.

Dose-effect studies: interspecific differences in UV sensitivity

Various marine and freshwater algae were tested for their sensitivity to chronic UV-B exposure using growth rate reduction as an overall indicator of UV-stress. Large differences in sensitivity were found between species. A comparison of a number of freshwater species demonstrated a generally higher UV sensitivity of desmids and diatoms as compared to green algae. The prymnesiophyte *Emiliania huxleyi*, thought to be an important bloom forming alga in temperate and boreal marine waters, was found to be very sensitive to UV-B, more than any other marine alga tested. Although marine pelagic diatoms differed considerably in their UV-sensitivity, a comparison with benthic diatoms, isolated from the Dutch Wadden Sea, revealed a significantly higher tolerance of the latter group. No general difference between pelagic diatoms and toxic dinoflagellates was found. Remarkably, a freshly isolated strain of a benthic diatom species did not have a higher UV-B tolerance than a strain that had been cultured in the lab for several years.

Reciprocity between UV dose rate and exposure time was tested in several algal species. The inhibition of growth rate was stronger at shorter exposure times (high dose rate) than at long exposure times (low dose rate). In other words, damage of chronic UV exposures was not only a function of dose. These results further stress the importance of applying accurate dosimetry and careful experimental setup.

Studies of mechanisms behind growth inhibition

In preliminary experiments photoinhibition did not explain the observed UV induced growth inhibition. Upon UV-B exposure, increases in cell dry weight were observed, while the light capturing capacity remained unchanged. This indicated that the photosynthetic apparatus is not the primary target under chronic UV exposure. Also the effect of low UV-B levels on pigment content, cell size, protein and carbohydrate content, and ultrastructure were investigated. UV induced growth rate reduction was typically accompanied by increases in protein content, pigment content and mean cell volume. These trends were stronger at higher UV-B radiation levels. However, at very high UV-B doses, when complete growth inhibition occurred, no increase in cell components were measured. These results were found to be very consistent when testing different species and various UV culture systems (see also below). The increases in cell dry weight, protein and carbohydrate content, pigment content and mean cell size hint at a UV mediated delay in cell division. In this view, low UV-B levels would arrest DNA synthesis through the occurrence of DNA damage, but not the biosynthesis of cell components, thereby arresting the cell cycle at the end of the Gl phase. This was later on confirmed by DNA content and DNA damage measurements in the marine diatom *Cyclotella sp.* (see above).

UV-B Effect studies using computer controlled Dynamic Light Regime

To realize an accurate and realistic dosimetry, a continuous culture systems with steering software was developed to simulate natural photosynthetic light and UV-B regimes simultaneously. The culture system was designed to allow exact quantification of UV-B exposures. High intensity light sources (PAR up to 2000 μmol.m-2.s-l) provide a realistic UV/PAR ratio. By computer controlled angular slat displacement of Venetian blinds natural sine-curve-like intensity courses for both UV and the visible part can be simulated, as well as the light regime for a superimposed vertical mixing. The combination of rectangular algal cultures and light sources allows accurate dosimetry. In this culture system the effect of UV-B on growth rate and other physiological parameters of the common green freshwater alga *Selenastrum capricornutum* was studied. During short term exposure experiments (transition from no UV-B to UV-B) only significant damage to the photosynthetic system could be detected. A reversed transition to no UV-B demonstrated recovery of the system. However, although with a lower midday level, a more balanced photosynthetic activity, comparable with the conditions in the absence of UV-B radiation, was observed after a prolonged exposure to UV-B radiation. The measured daily photosynthetic activity under chronic UV-B exposure poorly reflected the overall growth conditions. As shown in Figure 4.1, chronic UV-B exposure caused significant increases in mean cell dry weight. Also, mean cell volume and pigment and carbohydrate content increased as a result of UV-B exposure. These results, in combination with the photosynthesis measurements, once more hint at UV-B induced cell cycle arrest due to DNA damage rather than inhibition of the photosynthetic apparatus. These results also emphasize the incompatibility of acute and chronic UV effects: acute UV responses, such as those measured in short term oxygen evolution or ^{14}C incorporation experiments, might well underestimate chronic effects measured after several generation times.

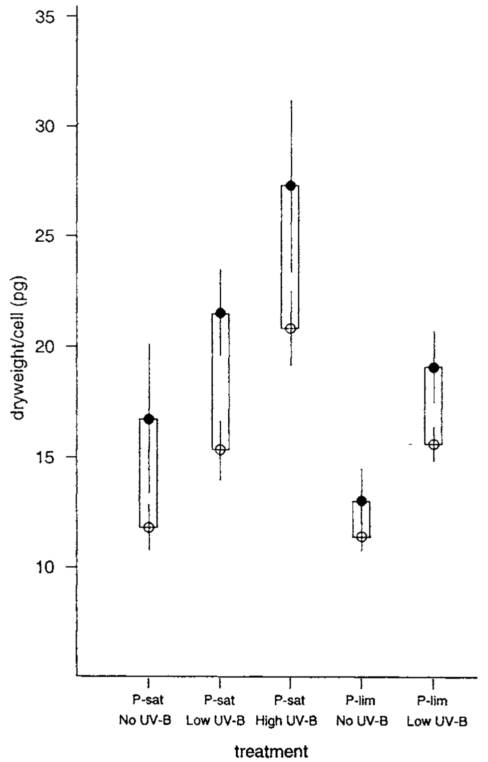

Figure 4.1
Steady State levels of Selenastrum capricornutum cell dry weight as a result of UV-B exposure. P-sat = P-saturated; P-lim = P-limited. Open circles: start of light period; closed circles: end of light period; Vertical lines through data points: standard deviations

DNA damage and repair

Very little is known about UV-B induced effects on microalgal DNA. This lack in knowledge is surprising, since UV effect studies on higher organised eukaryotic organisms, including man, are mainly focussed on structural changes in DNA. Different lesions can be induced by UV, of which thymine dimers are not only the most abundant photoproducts, but also typically formed as a result of UV-B exposure. A method was developed to detect this kind of cyclobutane dimer in single microalgal cells with a monoclonal antibody, following recent developments in skin cancer research. Detection and quantification of DNA damage is possible after fluorochrome labelling in combination with epifluorescence microscopy and flow-cytometry. A linear relationship was found between the applied UV-B dose and the amount of thymine dimers found in nuclear *Cyclotella sp.* DNA (Figure 4.2). Within a population of cells the amount of damaged cells, as well as the mean damage level in damaged cells increased with increasing UV-B dose. Kinetics of damage and repair have been studied at various low levels of UV radiation.

Simultaneous measurements of cellular DNA content showed a decrease in DNA synthesis during and after several hours of UV exposure. These results, in combination with the observed increases in cell size and cellular protein and pigment levels, indicate that the cell cycle is arrested at the end of the G1 phase. It was also found that the cell cycle can be resumed only after the damage is repaired. Additionally, a shift was observed towards G2 cells in populations of *Cyclotella* which had been exposed to UV-B for several days. This seems to be due to the fact that cells with a higher DNA content contain more thymine dimers, requiring more time and energy for repair processes. Determination of species-specific differences in the induction of damage and repair may explain differences in UV-B tolerance and therefore be useful for UV risk assessment studies with respect to shifts in natural populations exposed to a structural increase in incident UV radiation.

Action spectra

Action spectra determined so far were either based on the study of higher eukaryotic organisms or isolated cell organelles. Mostly monochromatic light instead of polychromatic light was used to establish these action spectra. For phytoplankton one general action spectrum has been described, based on short term photosynthetic rate measurements. Internationally, very little attention has been payed to the establishment of action spectra, probably because the description of an action spectrum is difficult and time consuming. A special culturing system was developed to study wavelength dependent chronic biological effects using whole organisms and polychromatic light. Using the marine diatom *Cyclotella* sp. as the test organism, tentative action spectra have been constructed for growth inhibition and DNA damage. Simultaneously wavelength dependent changes in physiological parameters were studied, such as protein and photosynthetic pigment content, and cell size. Both the DNA and growth inhibition action spectrum are very steep in the UV-B region of the spectrum and very much alike. Also, protein, pigment and cell size patterns showed high correlations with the action spectra. The results indicate that wavelength dependent UV-B effects on growth rate are strongly determined by DNA damage and thereby by changes in the cell cycle.

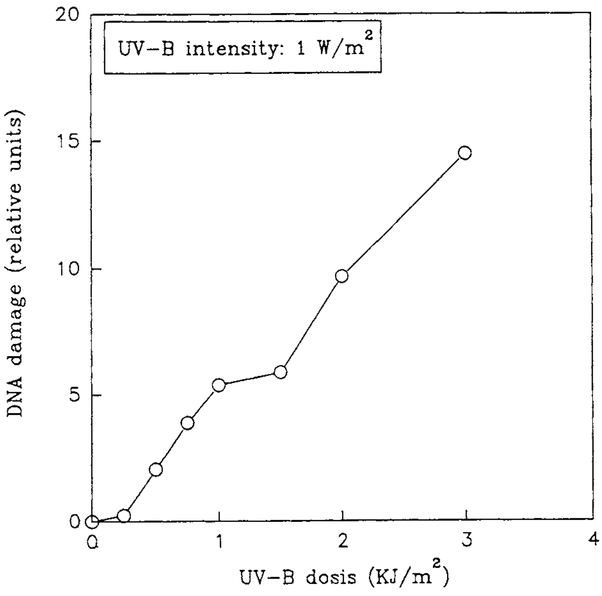

Figure 4.2
The formation of thymine-dimers in *Cyclotella sp.* as a function of UV-B dose. DNA damage (Y-axis) is expressed as the mean damage level in damaged cells within a population of UV-B exposed cells

UV-B effect model

To integrate the experimental results the development of a UV-B effect model has been started. The model structure is shown in Figure 4.3. To realize a global level the Atlantic Ocean has been selected as the model system. It has been chosen because of its pole to pole orientation, covering all climatic and latitudinal zones, both coastal and oceanic. Also, a substantial amount of literature data is available on the various subsystems. By the integration of a mathematical model with the effect study, an improved framework and structure is created for experimental research. The dynamic model is aimed at foodweb processes. Phytoplankton growth is regulated by nutrients, temperature, PAR and UV-B light, and zooplankton grazing. A distinction will be made between different algal and zooplankton groups, each with their own specific parameters. In this way shifts in the food web are included as combined effects of ΔDT , ΔCO_2 and ΔUV-B. Changes in primary and secondary production can be expected. The model will provide both science and politics with a tool to obtain an integrated picture of UV-B effects on aquatic ecosystems. Due to the technical complexity of the research and the rather unexplored field of UV-B effects on ecosystems, the development of the model (and the generation of experimental input data) will overrun the NRP I period. The expected uncertainties in the model will be quantified and reduced by means of a recently developed model analysis resulting in probability distributions for the parameters and variables.Using this model risk assessments will be made for different marine systems and UV scenarios.

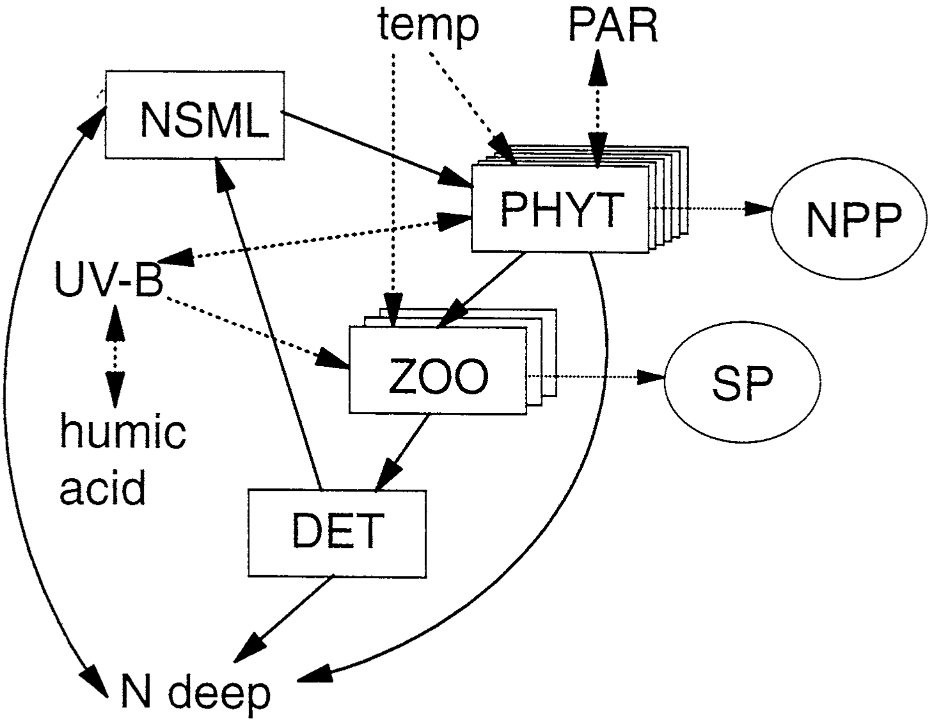

Figure 4.3
Schematic structure of the UV-effect model. NSML = Nutrient Surface Mixed Layer; Ndeep = Nutrient deep layer; PAR = Photosynthetic Active Radiation; temp = temperature; NPP = Net Primary Production; SP = Secondary Production; PHYT = Phytoplankton; ZOO = Zooplankton; DET = Detritus

4.5 Conclusions and perspectives of results obtained during NRP I

Increased exposure to UV radiation can affect aquatic ecosystems. Phytoplankton produces about 30% of the biomass on Earth, and thereby fixes a large proportion of the greenhouse gas carbon dioxide. Changes in the structure of the algal community may seriously endanger species higher in the food chain. A study of the effects of UV on marine ecosystems is especially relevant because changes in biological activity in the ocean have repercussions on global biogeochemical cycles, not only of carbon but also of sulfur, another element that is directly related to climate change through dimethyl sulfide emission from ocean to atmosphere.

During NRP I new insights were gained with respect to UV penetration in aquatic environments and UV stress phenomena after chronic exposure to low levels of UV-B. The results collected during this project will contribute to the knowledge of tolerance and selection.

The results obtained during NRP I have revealed that many species show growth inhibition even at ambient UV-B levels. Benthic algae isolated from the Wadden

Sea are less sensitive to UV-B compared to pelagic species. This is most likely due to genetic adaptation to the ambient UV-B levels impinging on mud flats during spring and summer. In contrast, marine pelagic species of clear open waters, such as *Emiliania huxleyi,* are very sensitive to UV-B. This indicates that pelagic algal communities will be affected more strongly by increases in incident UV-B radiation. Clearly, UV-B is a natural selective factor in plankton assemblages. Worrest (1983) already calculated that a UV-B free environment would increase global primary production with 12%. Since *Emiliania huxleyi* forms blooms in those areas where ozone depletion has been recorded (northern parts of the Atlantic Ocean), increases in incident UV-B radiation will strongly affect the performance of this organism in the field. As shown in Figure 4.4, which is a combination of experimental growth inhibition data and BED values derived from our radiation and penetration models, significant growth inhibition of Emiliania huxleyi due to UV-B will occur during summer at 52oN at the mean ozon level for this latitude: 50% growth inhibition at 5 m depth considering the DNA action spectrum (ED50(DNA) in Figure 4.4); 50% growth inhibition at 15 m when considering the Plant action spectrum (ED50(PLANT) in Figure 4.4).

The results have shown that short term effect studies done so far to estimate UV-B effects on microalgae give a considerable underestimation of UV-B effects: our chronic exposure experiments have revealed that short term photosynthesis measurements are not representative for chronic effects such as growth rate reduction. This means that the calculations of production decrease based on short term experiments of UV effects on photosynthesis in Antarctic waters (12% reduction of primary productivity, as estimated by American scientists) have to be reevaluated using parameters which take into account the chronic UV-B effects such as growth reduction. Our experiments also suggest that growth rate reduction is a more reliable and realistic parameter to be measured than the production measurements done so far: growth rate takes into account UV-B effects over several generations, and, moreover, growth rate is a resultant of all metabolic processes affected by UV-B, including DNA damage - certainly not only the photosynthetic process. UV-B effect modelling is in need of urgent adaptation with respect to this point. This will be realized in the second phase of NRP (NRP II).

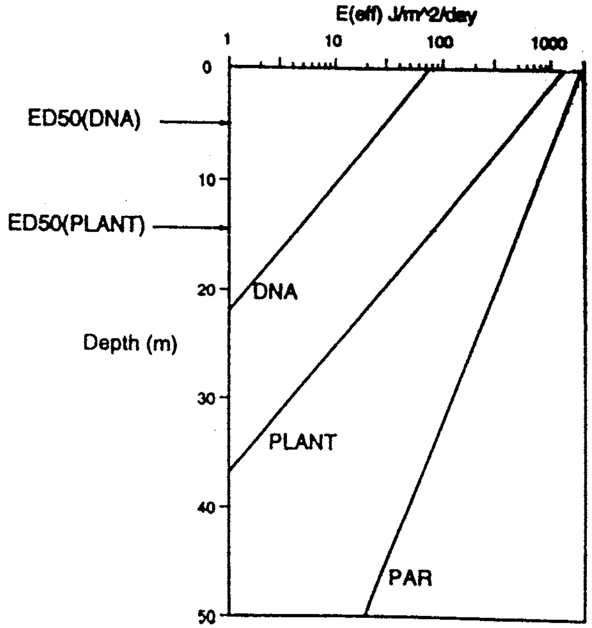

Figure 4.4
Calculated UV-B exposure for 15 June at 52°N and ozone thickness of 320 DU. x-axis: biologically effective daily UV-B dose (J.m-2.day-1); Y-axis: water depth (m); DNA: biologically effective dose calculated with Setlow's DNA action spectrum; PLANT: biologically effective dose calculated with Caldwell's Plant action spectrum. ED50(DNA): depth at which 50% growth inhibition occurs for *Emiliania huxleyi* considering the DNA action spectrum; ED50(PLANT): depth at which 50% growth inhibition occurs for *E. huxleyi* considering the Plant action spectrum

The results obtained during NRP I have indicated that DNA damage and its subsequent effect on the cell cycle plays a major role in UV-B mediated growth rate reduction. This approach is new in aquatic UV-B effect studies and further underlines the importance of studying UV-B effects over several generation times. DNA damage occurs at realistic UV-B levels in most tested species, and the ability to overcome DNA damage is now thought to be a primary determining factor of UV-B sensitivity in phytoplankton species.
A substantial body of evidence is presented during the project, indicating that UV-B affects the cell cycle of microalgae, causing an arrest in the G2 phase. This causes shifts in mean cell volume, pigmentation as well as the content of structural compounds and storage products. This will most certainly result in changes in the aquatic food web. Herbivore predators in aquatic environments will be affected by these changes because many herbivores rely on a defined food size spectrum, which is altered by cellular volume increases. Also sinking rates will be affected, resulting in a proportional increase in vertical carbon fluxes out of the euphotic zone.

Although refinements have to be made with respect to validation, the development of the radiation and penetration models have provided useful tools for the calculation of UV-B exposure to phytoplankton in situ. Furthermore, action spectra will furnish the assessment of biological effective dose rates in natural waters. The action spectra for DNA damage and growth can be incorporated in the radiation models after necessary fine tuning operations planned for the future.

The experimental results obtained during NRP I provide basic insight into the key parameters to be included in the model dedicated to risk assessment for specific marine habitats. Insight into the exposure of algae to chronic natural levels of UV-B and effects on the cellular level, to a great extent derived from the insights gained during NRP I, form a firm basis to establish first modelling on large scale. Within the coming years our UV-effect model can become operative (depending on NRP II funding). This simulation model is specifically focussed on the vulnerability of the entire Atlantic Ocean ecosystem for integrated climate change factors, including UV-B, temperature and carbon dioxide, and is so far unique within the international scientific community.

4.6 Cooperation with other research groups within and outside NRP

Within NRP framework:

- VU Amsterdam The Netherlands (Dr. J. Rozema): cooperation with respect to DNA damage measurements and modelling.
- AZU The Netherlands (Prof. J.C. van der Leun, Dr. F.R. de Gruijl): DNA damage method and action spectra.

Outside NRP framework:

- Laboratory of Radiation Research (at RIVM), The Netherlands: UV/Ozone data.
- NIOZ, The Netherlands (Dr. M.J.W. Veldhuis): Flow cytometric DNA damage measurements, Cooperation between NRP participant and NIOZ is being materialised in a joint Ph.D project dedicated to the effect of UV-B on oceanic picoplankters. This project (financed by NIOZ) will start in the autumn of 1994.
- LU Wageningen, The Netherlands (Dr. E. van Donk) UV-B effects on phytoplankon-zooplankton interactions.
- TNO, The Netherlands (Dr. L. Roza): DNA damage method development.
- RUG Plant Physiology Dept., The Netherlands (Dr. F. van Hasselt): General exchange of methods and equipment.
- University of Oldenburg (Prof. Dr. Th. Hüpner): Mesocosm experiments.
- University of Lund, Sweden (Dr. Nils Ekelund): DNA damage measurements in mesocosm experiments.

4.7 Effects of increased UV-B radiation on structure and functioning of algal communities in different climatic zones: risk of a global decrease in stratospheric ozone

Assessment

Among the innovating elements in this project are the prolonged exposures to enhanced UV-B radiation, extending over several generations of the organisms studied. This realistic experimental condition provides for the inclusion of processes such as repair of DNA damage, and adaptation. For the first time, attention is given to DNA-damage in algae, using techniques developed in Dutch research on skin cancer. Initial determinations were made of action spectra for some of the effects of UV radiation on algae; these show a strong involvement of the UV-B wavelength range.

This work is giving real contributions to the international efforts to predict the effects of increased UV-B irradiance on phytoplankton and the potentially important consequences for the global atmospheric.

5. INTEGRATION

Integration of the various topics is obviously desirable in a large national research programme. The interim evaluation by S&PA and HCG signalized that in the area of human health there were projects on malaria vectors and UV-B effects, but direct effects of climate on human health were not covered in the NRP. The latter should be included anyway in the integration efforts under Theme Integration.

The immediate reason for the lack of projects on direct effects of climate on human health was that there were no project proposals in this area. The reason behind this is probably that The Netherlands have a moderate climate, where extreme weather conditions are rare; death by heat or cold is practically unknown, and is unlikely to occur with the changes now within the range of expectations. Research into such effects is apparently not considered urgent, or expected to be rewarding.

The integration desirable was achieved in a different direction. The groups investigating effects of UV-B radiation on health, terrestrial and aquatic ecosystems joined forces, and standardized equipment, measurements and methods. Beyond the effects groups the contacts were extended to the groups making measurements of ambient UV radiation and those making integration models. The specifications of the UV-measurements were carefully adjusted to the needs of the effects studies. And the UV-B effects groups provided input for a longitudinal integration model. This model was developed outside, but in close connection with the NRP, and ranged from production and release of ozone depleting chemicals through atmospheric change to penetration of UV-B radiation and future incidence of skin cancer in The Netherlands; it is the explicit intention to include also other effects of UV-B radiation as soon as sufficient quantitative information will be available (Slaper et al.,1992).

6. RELEVANCE FOR POLICY MAKING

Even while only the skin cancer effects are sufficiently quantified to be taken into account in the integrated model mentioned, the results already have relevance for Dutch policy. The additional mortality from skin cancer, predicted on the basis of ozone depletions as expected during the coming decades well exceeds the limit of 1 death per million of the population per year (Health Council of The Netherlands,1994). This limit of 1 per million per year is considered the "maximal tolerable risk" in Dutch policy with respect to other environmental factors influenced by human activities, such as chemicals and iozining radiation.

The ozone depletion that will occur during the next 50 years in spite of successful international efforts to protect the ozone layer will, therefore, have at least one effect of relevance to Dutch policy. As skin cancer is the only effect for which such calculations can be made until now, this finding suggests that quantification of other effects, on health as well as ecosystems, is highly desirable. Some of these effects may will be more important than skin cancer.

The Health Council of The Netherlands is an advisory body to the Dutch Government in matters of health and of the environment.
The report on ultraviolet radiation from sunlight was to some extent a byproduct of the research activities in the NRP. The report was prepared by a committee of 14 scientists; 8 of them were also involved in the NRP. The Minister of Housing, Physical Planning and the Environment recently wrote a letter to the Chairman of the Health Council (letter SNV/27694009, dd. July 7, 1994), recognizing the value of the UV-report for Dutch environmental policy.

Likewise, the Dutch input to the new (UNEP, 1994) Assessment on Environmental Effects of Ozone Depletion results mainly from research sponsored by the NRP.

7. OUTLOOK: FUTURE WORK

The conditions for research on effects of increasing UV-B radiation are unsteady. Granting agencies tend to refer to each other for the funding. This is made even easier by the fact that the necessity of this research is contested from a variety of viewpoints:
a. The problem of ozone depletion does not exist.
b. The problem exists, but the biological impacts are negligible.
c. The effects on human health may be easily prevented.
d. The problem was already solved.
Each of these statements will be briefly discussed.

The problem of ozone depletion does not exist
There is a fierce publicity action to deny the problem of ozone depletion. It would be a fake problem, brought into the world by commercial interests. Moreover, the reasoning continues, even if ozone depletion would occur, it would not lead to more UV-radiation, and even if there would come more UV radiation, that would be beneficial rather than harmful (Maduro and Schauerhammer, 1992).

The message is pseudoscience, but for the media this message appears to have news value, more than the points of view of regular science which are fairly well-known by now. The counteraction is partly successful, at least in inducing question marks in public opinion. The potential danger is erosion of the public support for the efforts to protect the ozone layer.

The problem exists, but the biological impacts are negligible
This viewpoint is around in many forms. I quote one example, written recently by a scientist working in a related field. He suggests that the impacts of the expected increases of UV radiation cannot be very serious, because "an increase in yearly

effective UV of 15% at groundlevel is comparable with moving about 400 km nearer to the equator (e.g. Groningen-Maastricht) or moving to an altitude of 500 m compared to sealevel".
It appears useful to discuss this type of argumentation, as it occurs more generally.

The reasoning given can hardly apply to natural ecosystems. Seas and forests do not move appreciably. But even if the reasoning would be valid only for people, it would save a lot of work if it would be so easy to come to a conclusion on the health effects of increased UV-B radiation.

The conclusion may appear plausible to a person who did move 400 km South. His individual risk for a particular health effect may have increased from 1.0×10^{-4} to 1.3×10^{-4}, but he cannot possibly notice that. He may well be convinced that there was no change at all.
Multiplication of this zero-impression by the number of people in a population forms the basis for the suggestion that the risk of such moves is also insignificant for populations. The correct procedure would be, of course, to multiply the estimated increase in individual risk by the number of people in the population. Such estimated risks are, however, not yet available for most of the effects under consideration. But this is no excuse for following a loose reasoning which cannot even produce a valid approximation.

The risk of moves by entire populations cannot be checked directly, either, because such moves are practically impossible. The Dutch population could move to an altitude of 500 m only by going up into the air, and 400 km south only by invading Belgium and France. How can we know that the risk of such moves would be small?

The best indications in this area are produced by migration studies in epidemiology. These investigate how individual risks change by migration from, for instance, Western Europe to Israel or Australia. The results of such studies, together with many other data, form the basis for the risk estimates made for human health. Only from such quantitative estimations it can be concluded whether or not a certain change of UV-B radiation can have important effects. For many effects, further investigations are needed before such a conclusion is possible, but some well-founded quantitive estimations have already been made for skin cancer. These have lead to the conclusion that a 20 percent increase in the incidence of non-melanoma skin cancer is to be expected after serveral decades as a consequence of ozone depletion (Madronich and de Gruijl, 1993; UNEP, 1994). As these cancers already have the highest incidence of all forms of cancer in several light-skinned populations, this will cause a significant aggravation of an existing public health problem. That can not be talked away with pseudo-quantitative suggestions.

The effects on human health may be easily prevented
For effects of increased UV-B irradiance on human health, there is a special consideration. The UV-doses received by people depend not only on the irradiance outdoors, but also on their own behaviour. Any increase in solar UV-B radiation may be compensated by staying indoors more of the time and, when outdoors, by keeping in the shade, wearing hats and more clothing and by using sunscreens. It is

easier, therefore, to limit undesirable effects by influencing the behaviour of people than by policies requiring knowledge of the many possible effects, such as melanoma and non-
melanoma skin cancer, cataracts, suppressions of the immune system, etc.

This solution presupposes that it is feasible to influence the behaviour of people in the desired way. Experience until now gives no strong support to this assumption. Some success is reported only from Australia in recent years. That was achieved in an intensive campaign, launched under exceptionally favourable circumstances: the incidence of skin cancer in Australia is so high that almost everyone knows the problem in his own family. Branches of the Antarctic ozone hole passing over Southern Australia helped mobilize public attention and fear. Even there it is an open question if it will be possible to sustain this changed behaviour for long periods of time, that is, for at least several decades. That would be necessary to influence some of the long-term effects, such as squamous cell carcinoma and cataract.

In areas where light-skinned people live under less extreme conditions, it will be even more difficult to convince people that they should change their behaviour. There is certainly reason for trying it, which was recently recommended by the Health Council of The Netherlands, and is now being carried out by the Dutch Cancer Campaign. There may also be reason to do research on how to influence peoples' behaviour more effectively.
There is no reason, however, to think that this can be done instead of studying the effects of UV-B radiation. Epidemiological observations strongly suggest, for instance, that working indoors, which appears one of the most efficient ways of reducing the UV-exposures, actually increases the risk of melanoma. That may have to do with the irregularity of the remaining exposures. In any case it indicates that protection is not an easy overall-solution. Just as other preventive measures it requires knowledge, such as dose-effect relationships and action spectra of the various UV-B effects.

The problem was already solved
Prediction of effects of increased UV-B irradiance played an important role in the decision-making process leading to the international efforts to protect the ozone layer. The foundation was laid in the Vienna Convention for the Ozone Layer (1985). Limits to production and use of chemicals that damage stratospheric ozone were specified in the Montreal Protocol on Substances that Deplete the Ozone Layer (1987), and made more stringent in the Amendments of London (1990) and Copenhagen (1992). Practically all producer nations joined this process. According to the latest Amendment, production of almost all ozone depleting chemicals will be stopped by 1996.

This process cannot be speeded up any more, even if research would identify important new effects of increased UV-B radiation. This is reason for some policy-makers to decide that this type of research does not deserve high priority anymore. This has led to a decrease of funding, especially in the USA.

The reasoning given is convincing from the viewpoint of policy decisions on the Montreal Protocol. This is, however, a rather limited viewpoint. The banning of ozone depleting substances was a necessary first step. Even on the assumption of full and worldwide compliance with the agreements reached, the chlorine loading of the atmosphere will continue to rise for several years, and the ozone layer will be reduced for at least half a century. And anything less than full compliance will make the situation worse.

Policy makers, including those in the Dutch Ministry of Housing, Physical Planning and the Environment, are asking what will be the most important consequences, and what can be done to prevent or mitigate these. Giving answers to these questions requires much more knowledge than answering the initial question, of whether or not depletion of the ozone layer would have effects important enough to justify action to protect the layer.
Organized science has expressed itself clearly on this point. The International Council of Scientific Unions has formed a Scientific Committee on Problems of the Environment (SCOPE). SCOPE paid special attention to the effects of damage to the ozone layer that will persist for a long time in spite of protective action. It produced two reports, "Effects of Increased Ultraviolet Radiation on Biological Systems" (1992) and "Effects of Increased Ultraviolet Radiation on Global Ecosystems" (1993). The potential impacts are considered of such importance that the scientific community is urged to increase its efforts: "This new stage of quantitative questions will require full utilization of existing research capacity, and a major expansion in this direction is essential" (SCOPE 1992, p. 25).

The United Nations Environment Programme (UNEP) took a leading role in the international efforts to protect the ozone layer. It knows well what was achieved so far. But it does not support the notion that effects research fulfilled its function, and can have lower priority now. In its latest report on "Environmental Effects of Ozone Depletion" (1991), it calls for better funding of research on effects of increasing UV-B radiation: "Urgent problems are still waiting to be addressed" (UNEP, 1991, p.i).

Actual developments
The various conflicting views about the urgency of research on effects of increased UV-B radiation play a role in all communities, and cause great variations in funding.

Research efforts in the USA, which provided a large proportion of the information in earlier years, have decreased. The European Community, on the other hand, started late but increased its efforts in recent years in the research programme "Environment". Several European countries have national research programmes going.

In The Netherlands, NRP-I has given a real boost, roughly doubling the research volume in this area. With the changing philosophy, NRP-II putting emphasis on systems rather than climate factors, the support may well decrease again. This would be a loss for the generation of much needed knowledge, and a pity of the investments in equipment and know-how during NRP-I. Other sources of funding on this scale are not available in The Netherlands.

8. CONCLUSIONS

The projects on effects of increasing UV-B radiation in NRP-I have produced valuable contributions in their areas (section 3 of this assessment).

With respect to the importance of increasing UV-B radiation the following conclusions appear justified:
a. In the area of human health, UV-B radiation has many influences. The UV-B increases as expected even under the most favourable scenario of ozone depletion do have health consequences relevant to Dutch policy.
Uncertainty about other potentially grave impacts calls for intensive studies.
b. In terrestrial ecosystems, the importance of the UV-B irradiance is very different for the various subsystems. The NRP-researchers made progress in identifying those sub-systems where UV-B irradiance is important.
c.In aquatic ecosystems the UV-B irradiance is likely to be an important factor. The effects of increased UV-B radiation also have feedback on the atmosphere.
The projects on UV-B effects benefited from mutual interactions, with respect to equipment, measurements as well as photobiological aspects. The interactions with the groups making measurements of ambient UV radiation and integration models for effects may be further intensified.

The new philosophy for NRP-II, with its emphasis on systems rather than climate factors, may lead to a spreading of UV-B research over different Themes. In that case, some form of interaction in the UV-B sphere should be maintained. It would be a real loss if this new philosophy would lead to cutting out the important study on effects of UV-B radiation on human health. In The Netherlands UV-B radiation probably has more serious impacts on human health than any other climatic factor.

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