HELLE

Health effects of low level exposures

Gezondheidseffecten van lage blootstellingniveaus

Aanbiedingsbrief Nederlands

Aanbiedingsbrief Engels

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Health Council of the Netherlands

Gezondheidsraad

to

the Minister of Housing, Spatial Planning, and the Environment

aan

de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer

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Contents

Chapter 1

The nature of the problem

The Health Council is closely involved in establishing the scientific foundation of exposure limits for substances and radiation in order to protect public health. Through the years, the Council has contributed to the formulation of principles and procedures, both for carcinogenic and for noncarcinogenic agents. As a rule, the discussion with regard to the derivation of health-based recommended exposure limits centres around the appropriateness of extrapolation methods (What can be inferred from data on high exposure levels and on experimental animals?). Generally speaking, there is a lack of direct information on the health effects of low levels of exposure. Effects at these levels cannot usually be detected by means of traditional animal experiments or epidemiological research. The capacity of these analytical instruments to distinguish between ‘signal’ and ‘noise’ is inadequate in most cases. Annex B of this report contains a brief outline of the difficulties and the established methods for tackling this problem.

In spite of this, the hope exists that the posited weak signals, if they are indeed present, can be detected by other means. The search will have to take place on a deeper level. In other words, an effort must be made to discover what occurs at underlying levels of biological organization when organisms are exposed to low doses of radiation or substances. Molecular and cell biology provide various methods and techniques which give an insight into the processes within the cell. This results in an increase in the knowledge about the molecular and cellular effects of exposure to agents, or stated differently, the working mechanisms which form the basis of the health effects. Last year, the Health Council considered that the time was ripe to take stock of the state of

knowledge in this field. To this end, an international working conference was held from 19 to 21 October 1997, entitled ‘Health Effects of Low Level Exposures: Scientific Developments and Perspectives for Risk Assessment’.

The central question was the extent to which the sometimes fast-growing knowledge about molecular and cellular effects offers the desired basis for extrapolation. Against this setting, a number of more specific questions which have been hotly debated for some time were also addressed. One of the primary questions concerned the traditional but increasingly questioned division between stochastic and non-stochastic working agents, and the corresponding division between exposure-effect relations without a threshold and with a threshold (see Annex B for a concise explanation). Thoughts were also exchanged on what is often referred to as hormesis: the notion that low levels of exposure could actually improve health. For the purpose of illuminating the many aspects of these issues, experts from a number of areas were invited. In addition to this, three agents were selected to serve as points of crystallization for the general debate: ionizing radiation, ultraviolet (UV) radiation and dioxins.

The present report calls attention to a selection of issues which emerged during the discussions on the above-mentioned central topic. Various more detailed questions and the wider context of the points considered are described at greater length in the enclosed conference report (Annex C) and in the background documents attached to the report (Annexes D and E). What follows is a series of considerations regarding the scientific basis for the derivation of recommended exposure levels, viewed in the light of current procedures and against the background of the work of the Health Council. In the preparation of the following comments and recommendations, various Dutch experts have been consulted (see Annex A).

Chapter 2

The state of knowledge

The participants were of the opinion that it would not be possible to formulate a general answer to the central question of the conference. Relatively speaking, a great deal is known about the working mechanisms of some agents, including ionising radiation, UV radiation and dioxins. Even with this knowledge, numerous problems stand in the way of achieving a far-reaching quantitative model for exposure-effect relations based on molecular and cell biology. For example, there is still only limited insight into how diseases and disorders which may partly be caused by the agents referred to come about. Even in cases where the molecular-biological foundation has been discovered to a large extent, some stages in the process from events within the cell to the manifestation of health problems remain a mystery. During the working conference, it was pointed out on several occasions that, in order to achieve a sound understanding, various levels of biological organization must be taken into account. As crucial as the study of molecular-biological processes may be, research with a more physiological orientation is just as important. On the other hand, the above-mentioned problems concerning the causes of disease can be put into perspective by realizing that it need not be essential to know all the details of every step of the process. Imagine that certain molecular or physiological biomarkers (more of which later) could be clearly linked to certain forms of exposure and health effects. In such a case, a detailed description of the intermediate process would be superfluous in terms of risk assessment. This is not to say that such information will not be extremely valuable for other purposes, such as the development of medical interventions.

If one examines pathogenetic processes from the perspective of risk factors, further difficulties present themselves. The conference participants pointed out that a whole range of phenomena can occur within a cell under the influence of xenobiotic agents. These include changes in gene expression, mutations and death of the cell due to apoptosis (programmed cell death) or necrosis (other types of cell death). It is also possible that such interactions do not leave clear traces behind. In many cases, not enough is known about the changes which can be brought about by specific agents. Consequently researchers often find themselves in the dark as to the possible health effects of these alterations, whether this means an increased risk of cancer, the acceleration of the ageing process or the disturbance of certain organ functions. Meanwhile there is yet another obstacle which affects this entire process: the usually unknown relationship between cellular processes (and their resulting effect) and the degree and rate of exposure.

There are therefore a multitude of questions, but we generally possess very little information with which to formulate answers, even in cases where general information on molecular and cellular processes is readily available. Mechanistic modelling of exposure-effect relations looks like remaining an unattainable goal in the immediate future, or at least modelling of the entire range of pathogenetic processes. However, an ongoing stream of knowledge about certain subprocesses of exposure to specific xenobiotic agents is available. As already indicated, this can sometimes be sufficient for assessing risk.

Interesting developments are taking place in such areas as toxicokinetics and toxicodynamics. By finding out how substances behave when absorbed into the body, it is possible to obtain a clearer picture of the biologically relevant (effective) exposure. With the help of so-called PBPK/PD models (PBPK/PD: *physiologically based pharmacokinetic and pharmacodynamic*) toxicologists are attempting to describe explicitly and systematically the associated distribution and metabolic processes. In the last few years, for example, a great deal of research has been done with dioxins. The validation of these kinds of models is generally recognized as a research priority and it is expected that approach will reduce such problems as ‘animal to human’ extrapolation.

An extension of the developments just outlined is the promising research into biomarkers for internal exposure and for high susceptibility. This could mean that people with certain genetic characteristics could be adversely affected by exposure to certain agents at an earlier stage or to a greater extent. If such biomarkers are traced and used successfully in phenomenological research, this will increase the statistical power of these analyses. Biomarkers for early effects, that is to say effects which precede the manifestation of health problems, will probably be longer in coming.

Trends in the field of transgenesis (the transplantation of desired hereditary characteristics into the genome of a laboratory animal) also look very promising. By deactivating target genes in mice, it is possible to disengage one or more specific cellular processes, such as DNA repair and metabolism of chemical substances. In this way it is possible to analyze how such processes influence the effect of exposure to xenobiotic agents. The strong increase in the susceptibility of some mutated mice to certain substances also enables the direct measurement of the effect of low dosages. In addition to this, transgenic mice with sensitive systems for the detection of mutations may be able to increase our insight into the effect of exposure to genotoxic agents in the near future.

Another important consideration is the ongoing progress in the field of information technology which facilitates simplification and refinement in the analysis and synthesis of all kinds of data. The conference heard that possibly too little use is still being made of the existing opportunities in this field. The statistical processing of the findings of animal experiments and epidemiological research, where possible enriched with information on working mechanisms, can provide further indications on the degree of uncertainty governing the establishment of exposure-effect relations and the derivation of recommended exposure limits. Such methods of analysis can sometimes offer a definitive answer as to the probable existence of a threshold dose for the occurrence of certain effects.

The actual circumstances in which exposure takes place can be seen as a separate issue. In practice there is always a combined influence of a broader or narrower spectrum of endogenous and exogenous factors, with partly corresponding working mechanisms. One example is the production of so-called free radicals (certain reactive molecules) by the normal oxygen metabolism and by exposure to ionising radiation. At the conference, two questions were raised with regard to this issue.

Firstly, it may be worth calling into question whether it is useful or meaningful to derive exposure-effect relations for individual agents. This immediately gives rise to a second question, namely which basic principles should be brought to bear when determining the relations between combination-exposure and health effects. The deliberations at the conference failed to produce a theoretical direction in which a solution should be sought.

The second question concerns the possibility of hormesis. The idea that exposure to specific agents under certain circumstances mobilises certain reaction mechanisms which reduce the net damage caused by combination-exposure is not discounted by some researchers, while others even consider it plausible. In the general view at the conference, however, there was as yet no conclusive evidence to support this claim.

Chapter 3

The implications for risk assessment

The arguments above lead to the conclusion that the present system of assessment can be refined on certain points and under certain conditions. Developments with regard to elements such as PBPK/PD modelling, biomarkers for variations in susceptibility and modelling methods, present opportunities in terms of a firmer foundation for elements or modules which occupy a place within the current system. These might take the form of better founded safety or extrapolation factors, which would for instance allow possible differences between and within species to be taken into account. Ideally, such factors can be replaced by models or sub-models which provide an explicit description of the variations. More generally, it is to be expected that the relationship between the components of the so-called integral toxicity profile, as described in the Health Council report 'Toxicology-based recommended exposure limits' (1996/12), can be mapped out in greater detail with the help of the analyses referred to here.

As regards the circumstances under which deeper analyses appear justified, attention should be paid to the efficiency of the risk assessment. Thorough analyses like those alluded to are labour-intensive and costly to carry out and it is worth considering their initial application to agents with a societal priority by way of a trial. Criteria such as the plausibility of harmfulness at exposure levels expected in real life situations, the size of the population exposed, the seriousness of the effects, the possibility of risk reduction and the extent of the economic interests involved, could all be useful elements in the selection of these agents. A selection process of this kind is also of importance for other reasons: the risk assessment of existing chemicals on the

European market is proceeding rather slowly. Time and resources will need to be set aside in order to bring about improvements in this respect.

When addressing the depth and appropriateness of risk assessment issues, the well-orchestrated input of various experts is a crucial factor. Examining each case in turn, and in close consultation with each other, they will have to consider which model best describes the whole picture formed by the available details. At this stage, it is difficult to make a definitive statement about the general relevance of such models. The conference sessions on ionising radiation, UV radiation and dioxins illustrated this problem (see Annex C). In any case, it is not possible at present to produce general recommendations with regard to basic principles for mechanistic modelling or with regard to the influence of homeostatic control processes. In short, experience will teach us the ways in which and the speed with which the present system of assessment will lend itself to refinement.

In the Health Council's 1999 Working Programme, five topics closely related to the issues outlined above are included under the heading 'Principles for health-based recommended exposure limits': (1) the drawing up of an integral toxicity profile; (2) the use of epidemiological data in the drawing up of such a profile; (3) the application of a so-called benchmark dose approach (the benchmark dose is the lower statistical confidence limit of an exposure level corresponding to a specified response level); (4) the use of safety margins and (5) dealing with combination exposure. In the Netherlands, research into a number of these topics is taking place at university departments, in independent and governmental research laboratories, and in industry. The often intensive cooperation between these institutions and their equivalent organizations abroad will certainly benefit the quality and the efficiency of risk assessment in the Netherlands.

Annexes

Annex **A**

Drafting of the report

The present report has been prepared by Eert Schoten, scientific secretary at the Health Council, after consultation of the following experts.

- dr B Brunekreef; professor of health studies; Agricultural University of Wageningen; The Netherlands
- dr VJ Feron; professor of biological toxicology; University of Utrecht; The Netherlands
- dr JHJ Hoeijmakers; professor of molecular biology; Erasmus University Rotterdam; The Netherlands
- dr PHM Lohman; professor of radiation genetics and chemical mutagenesis; Leiden University Medical Center; The Netherlands

Annex **B**

Brief explanation of a number of concepts

For the purposes of convenience, the term *exposure-effect relation* is used throughout this report. Strictly speaking, the consequences of exposure can be divided into *effect* and *response*. The report 'Toxicology-based recommended exposure limits' (1996/12) defines an *effect* as the specific reaction of an organism upon exposure to a xenobiotic agent, while the term *response* refers to the fraction of organisms in the exposed population in which a certain effect occurs.

Whether we are dealing with *exposure-effect relations* or *exposure-response relations*, the nature of these functional links is increasingly difficult to detect the lower the exposure level becomes. Expressed in more concrete terms: if the strength of the 'signal' decreases linearly in relation to exposure, the size of the population studied has to increase quadratically to allow 'signal' to be distinguished from 'noise'. In practice this entails that capacity for detection — the *statistical power* — of animal experiments and epidemiological research will be inadequate from a certain point. Statements about the relations in question must therefore be based on certain assumptions: in other words, it becomes necessary to *extrapolate*. One of the primary assumptions concerns the working mechanisms which underpin the health effects. In the case of a *non-stochastic* or *deterministic* working mechanism, it is assumed that the health effect will only occur above a certain level of exposure (*threshold dose*), above which the damage increases as the exposure increases. In cases where the agent has a *stochastic* working, it is generally assumed that such a threshold dose cannot be established and that the chance of an effect from the zero point will increase as the exposure increases.

This distinction is being called into question more and more. According to some experts, agents with a non-stochastic working can sometimes be best characterized by relations without a threshold: certain effects on the nervous system provide good examples of such cases. Others feel that there is evidence to support the possibility of *hormesis*: that health improves under certain exposure processes. These and other related questions were the motivation behind the organization of the ‘Health Effects of Low Level Exposures’ conference.

Annex C

Conference report

Introduction

The Health Council of the Netherlands, which informs the government and parliament on the current level of knowledge in the field of public health, has a long tradition of assessing the health risk of low levels of exposure to physical and chemical agents. The Council frequently discusses how scientific data can be utilized to shed light on the possible effects of these low level exposures. Epidemiological research into the health status of populations exposed to low levels of radiation or chemical substances is in principle the most direct approach for making the risk assessments in question. But often its results, if available, have insufficient power. Therefore, one must resort to other, less direct but potentially relevant, types of evidence, such as information on biochemical processes and experimental animal data. Explicit, or sometimes tacit, answers to the questions involved result in recommendations to use particular extrapolation models, which make it possible to deduce what happens at low levels of exposure in the absence of direct data.

Because standard setting and protection measures frequently depend on the chosen extrapolation models, it is of great societal importance that these models be based on the best available evidence. This raises the question of when and how new scientific insights should be incorporated in the process of risk assessment. Particularly, developments in molecular and cell biology generate a continuously increasing set of data on the modes of action of agents. Many experts hold that risk analysts should develop techniques to take these data into account.

In order to explore the possibilities and limitations of what may be termed 'evidence based risk assessment', the Health Council decided to organize an international working conference, entitled 'Health Effects of Low Level Exposures: Scientific Developments and Perspectives for Risk Assessment' . Co-sponsors were the Dutch Ministry of Housing, Spatial Planning, and the Environment and the European Commission. The meeting took place in Lage Vuursche, the Netherlands, from 19 till 21 October 1997. This report summarizes the introductions given by participants and subsequent discussions.

The working conference focused on three broad topics:

- the state of knowledge about deleterious effects and defence mechanisms occurring at low levels of exposure
- the implications of those insights for risk assessment procedures, and
- the types of research needed on a priority basis

Three cases served as crystallizing points for the exchange of ideas: ionising radiation, UV radiation, and dioxins. The main rationale for this choice was that relatively much is known about their effects and modes of action. Participants were asked, however, to discuss issues concerning these cases with a view to the general questions mentioned above.

The report also contains two background documents which were distributed in advance. In the first (see Annex D) a general survey was given of the major scientific and societal aspects of the ongoing debate about low level exposures. The second (see Annex E) provided some topical information for the sessions of the working conference and contained a number of additional, and more specific, questions related to its main theme. The conference programme is reproduced as Annex F. The members of the Scientific Advisory Committee and the conference participants are listed in Annex G.

On the basis of the findings of this conference the Health Council will advise the Dutch government on principles of risk assessment for low level exposures.

Session I Heuristic overture

The introductory contributions of this session provide some general ideas and methodological considerations which may help develop a conceptual framework for assessing the possible health effects of exposure to low levels of environmental stressors. A recurring theme and major issue is how to integrate the influence of a particular agent and the effects of simultaneously operating endogenous and exogenous ('background') factors. Evolutionary biology and biogerontology seem to be among the disciplines attempting to give an answer to this problem. At least they tend to favour

systemic or homeostatic concepts in stead of reductionist or linear cause effect schemes. Some integrative principles of order and control are briefly touched upon. In this connection one of the important and sometimes hotly debated topics is a class of phenomena referred to as hormesis: a beneficial response to low doses.

Opening address (Knottnerus)

Dr Knottnerus, vice president of the Health Council, opens the working conference and welcomes the participants. After briefly sketching the mission and organizational structure of the Council and the background of the conference, he draws attention to some topics for discussion. Nowadays chemical and radiation risk assessment mainly relates to low levels or low rates of exposure. One usually faces major methodological difficulties in determining the — potential — health effects of such types of exposure. For example, there may be very little contrast between normal ('background') exposure levels and additions due to a specific practice. A similar problem may hold for the measurement of potential increments in response, e.g. in incidence or prevalence rates. Moreover, some effects can only be detected after many years of observation. Cancer and genetic disorders are a case in point. The possible influence of other exogenous and endogenous stressors further complicates things. Dealing with these difficulties is a challenging task, especially given the fact that susceptibility to agents varies across the population and repair mechanisms and adaptive responses play a role as well. Dr Knottnerus hopes that the participants, with their different fields of expertise, are able to make useful recommendations on the key topics of the conference.

Aim and structure of the conference (Schoten)

Mr Schoten presents an overview of the relevant points in the two background documents (see Annexes D and E). The aim of the conference is to discuss the possibilities and limitations of evidence based risk assessment. What counts as evidence? How can it be utilized in risk assessment? And what kind of evidence is most needed? With respect to these and similar questions he points to a number of problems. On the one hand, there is an ever increasing set of data on the effects of all kinds of agents, relating to various levels of biological organisation (molecular, cellular, intercellular, organismal). Yet integrating these various types of evidence into a single exposure effect model appears to be far from easy. Often only part of the available information is used in deriving exposure effect relationships. A major issue is whether generic recommendations can be made concerning methods of extrapolation given this variety of data. On the other hand, risk managers and governmental organisations show a regular interest in faster risk assessment procedures. That wish

may be at right angles to an in-depth analysis and synthesis of the available evidence. Reflecting upon this somewhat paradoxical situation, Mr Schoten advocates a differentiated approach to the development of principles for risk assessment. For instance, the thoroughness of an assessment could be made to depend on the societal impact of the decision in question. Also, ways of grading evidence could be useful for a decision support system.

Low level effects from a theoretical perspective (Doucet)

Hormesis is a term used to describe an hypothesized phenomenon, namely that at low exposure toxic agents turn out to be beneficial to organisms in some respects. Dr Doucet examines this phenomenon at a theoretical and general level. He is interested in the kinds of mechanisms which can produce non-monotonic exposure-effect curves. One may expect that hormesis is the outcome of some sort of control action. A feedback system, where the body's counteraction is triggered by the effects caused by the toxicant, seems to be the most plausible candidate from a biological point of view. If one thinks along these lines, one comes across a paradox. Consider a toxic agent whose only effect is to kill particular cells. Exposure to this toxicant will generate a response from the homeostatic control system involved, but the new equilibrium at which the cell population settles, will inevitably be below the original level. However, Dr Doucet thinks this argument disregards other possibilities. He wants to demonstrate that hormesis is possible in the presence of feedback control, provided that certain conditions are satisfied. To this end he formulates a model with two state variables, viz. a population of physiologically active target cells and a population that produces these target cells. Again it is assumed that the toxicant itself has only a negative effect. This excludes situations where hormesis can be attributed to a shifting balance between beneficial and deleterious effects. Dr Doucet's analysis shows that under particular conditions for the intrinsic growth rate of the producer cell population, the model system achieves the required behavior, i.e. hormesis and stability.

Low level effects from the perspective of evolutionary biology (Kooijman)

According to Dr Kooijman it is essential, but far from easy, to test purely theoretical ideas like those of Dr Doucet's against observational data. He gives a brief introduction to the so-called Dynamic Energy Budget (DEB) theory, which attempts to do just that. The DEB model quantifies the fluxes of energy through organisms as they change during life history. Three stages are distinguished: the embryo (which does not eat, although it does consume), the juvenile (which eats but does not reproduce), and the adult (which eats and reproduces). Energy is used for competing physiological

processes, such as growth, maintenance, and reproduction. The rules for uptake and use of food provide an explanation for a variety of suborganismal phenomena and for effects on populations and ecosystems. Dr Kooijman thinks the DEB model can also be used to specify in a quantitative way how the energetics interferes with the uptake and effects of non-essential or toxic compounds. He mainly focuses on ecological risk assessment, but assumes that some considerations may be relevant to human toxicology as well. Most ecologists tacitly accept that organisms can cope with varying concentrations of any particular stressor, because they evolved in a chemically varying environment. The boundaries of this tolerance range appear to differ, however, depending on the stressor and on the physiological process. For instance, the upper boundary may be zero, which implies that even very small exposures, or rather tissue concentrations, have an effect or induce an effect with a certain probability. As a first approximation, the effect size is a linear function of the tissue concentration when it exceeds the tolerance range. Dr Kooijman supposes that for most compounds the upper boundary is positive and the lower boundary equals zero, because they are not necessary for life. Essential nutrients are an exception, with lower boundaries exceeding zero. The poorly understood process of aging is only of secondary relevance to the DEB model in its present form. Yet, descriptions of survival data where aging can be assumed to be the major cause of death seem to call for an extra integration step, which points to DNA. Dr Kooijman suggests that free radical activity (which seems to cause partly irreparable damage to DNA) could provide the clue to the relationship between age specific survival probability, life span, and energetics. The way aging is treated within the DEB model closely links up with mutagenic effects, particularly if the free radical mechanism is correct. According to dr Kooijman, mutagenic compounds have about the same effect on organisms as free radicals. As a consequence, mutagenic effects might be studied by changing aging acceleration.

Low level effects from the perspective of biogerontology (Vijg*)

Dr Vijg makes some comments on theories of aging, in particular on the role of oxidative damage to macromolecules as a mechanism of aging. This idea has a history that goes back some forty years, with papers on the commonality of mechanisms of oxygen toxicity and X-irradiation. Today techniques and assays are available to investigate the relationship between mutagenesis and aging at the molecular level. Studies indicate that protein and DNA oxidative damage substantially increases during aging. According to Dr Vijg, this phenomenon is most probably due to an increase in the rates of oxidant generation. No consensus has emerged as to whether or not the

* Dr Vijg was willing to substitute for Dr Kirkwood who had to cancel his participation at the last moment.

efficiency of antioxidant defences and DNA repair declines during aging. Neither has it as yet been determined whether oxidative damage is a somewhat random phenomenon or whether there are specific and critical targets of such damage. The second mechanism might be a contributory factor, given the predictable nature of age-related physiological changes and life spans of different species.

Possibilities and impossibilities of environmental epidemiology (Brunekreef)

Compared with experimental research in the field of toxicology or biogerontology, epidemiology has both limitations and advantages. Dr Brunekreef starts by mentioning some of its major problems. It is difficult to eliminate or minimize measurement errors and the influence of confounding factors, especially in the case of environmental epidemiological investigations, where the potential effects under analysis are small and confounders are numerous. On the other hand, epidemiologists directly study the endpoints that matter most, such as physiological effects and various disease outcomes. In his opinion, utilizing biomarkers offers good prospects for diminishing the gap between epidemiological and experimental research, but similar methodological difficulties will remain. Still, epidemiological studies may have sufficient power to detect small effects due to low levels of exposure to environmental stressors. Dr Brunekreef takes air pollutants as a case in point. Time series analyses of the correlation between fluctuations in the daily concentration of major air pollutants (such as particulate matter and ozone) and the daily mortality rates reveal a linear exposure-effect relationship without any apparent threshold, let alone a beneficial or hormetic response.

Discussion

Synthesis of biological subdisciplines with partially comparable approaches to explanatory descriptions or experimental methods, e.g. developmental and evolutionary biology or oncology and biogerontology, is gaining ground. Although the participants at the conference expect that such scientific trends in the long term enlarge the set of analytical instruments for risk evaluations, they believe that at present there are hardly any opportunities for risk analysts to benefit from these developments. No clear-cut guiding principles exist to incorporate ideas like ‘homeostatic control’ and ‘reserve capacity’ into chemical and radiation risk assessments. Neither can controversies about the possibility or plausibility of phenomena such as hormesis be solved with an appeal to these generally fuzzy concepts. Most of the participants believe that there is no hard evidence to support the idea of hormesis.

The discussion also makes it clear that there is no simple and uniform correlation between events at suborganismal levels and physiological or pathological effects. It is true that particular biochemical shifts might be predictive of disease outcomes, and molecular biology shows impressive success, but the physiological approach has its own merits and should not be neglected. Some participants point out that, moreover, the results of traditional epidemiological investigations may sometimes be quite adequate to risk assessment and risk management.

Session II Ionising radiation

Introduction (Bridges)

Relatively much is known about the biological and health effects of ionising radiation and about its mode of action, at least in comparison with most chemical agents. In addition, ionising radiation affects cells in a simple fashion, because metabolic processes do not play a role. Despite this favorable situation, however, many problems with respect to the effects of low doses and low dose rates still await a solution. According to Dr Bridges, emerging data are continuously shaking the radiobiological community out of complacency. On the one hand, there is experimental evidence that radiation may stimulate particular repair mechanisms. On the other hand, some studies indicate that one energy loss event can trigger more than one negative effect in cells. It is important to determine when such data are sufficient to take them into account in radiological protection.

Significance of data on repair mechanisms

Potential contributions from biomarker epidemiology (Cox)

Dr Cox considers the basic tumorigenic processes and the stages where ionising radiation appears to act, taking colon carcinogenesis as his example. Animal experiments and evidence from biochemical, cytogenetic and molecular studies suggest that neoplastic initiation is the key stage that is targeted by low doses of ionising radiation. The data are consistent with a monoclonal mechanism of tumor development that does not differ in a discernable fashion from that of a ‘spontaneous’ tumor. Tumor-suppressor gene loss is likely to be a factor of major importance in radiation oncogenesis. Other types of mutations may be contributing factors as well. According to Dr Cox, even a single radiation track traversing the nucleus of a target cell can generate a tumor initiating mutation, albeit at a very low frequency. In his view this implies that, at the level of DNA damage, there is no basis for the existence of a

threshold dose below which the risk of tumor induction will be zero. To defend this conclusion, he addresses the other side of the picture, viz. the influence of repair mechanisms. He makes a distinction between single strand and double strand DNA damage. The former arises spontaneously at a high frequency in the cell due to endogenous metabolic processes. Many experts argue that this form of DNA damage is repaired in an error-free fashion and does not make a significant contribution to cancer risk. However, double strand breaks, which appear to be a very minor component of spontaneous damage but can be efficiently induced by ionizing radiation, will not all be repaired correctly. Even at low doses some residual damage should be anticipated at the molecular and organismal level.

Next, Dr Cox discusses other potentially protective processes relating to the various stages of carcinogenesis and their corresponding levels of biological organization. There is some evidence that low dose radiation may induce or activate cellular defence systems: the so-called adaptive response. Three possible mechanisms have been suggested: additional DNA repair, induction of radical scavenging pathways, and subtle effects on cell cycle progression, which facilitate repair processes. Dr Cox thinks the last possibility is the most probable. But data relating to these responses and their relevance to neoplastic processes are insufficiently developed and understood to provide a sound basis for the judgement that carcinogenic response at low doses and low dose rates is likely to have a non-linear component, which might result in a dose threshold at the organismal level. In his opinion similar considerations apply to programmed cell death (apoptosis), terminal differentiation (to a non-dividing state), and immune surveillance: they have yet to be adequately described and remain contentious scientific issues with respect to their effects on carcinogenic response at low doses of radiation. Dr Cox concludes his introduction by making a remark on the prospects for molecular epidemiology and on individual cancer susceptibility. The development of molecular biomarkers is based on the mechanisms of action of the agent in question. Currently, it is difficult to determine the role of tumorigenic agents through mutational signatures present in a given tumor. However, genetic marker studies may be expected to improve and refine the ability to identify cancer susceptible populations by searching for specific germline mutations. In principle this approach could increase the power of certain epidemiological investigations.

Insights into adverse effects (Bridges)

Adaptive responses receive a lot of attention these days, but other unconventional effects should be taken into account as well. Dr Bridges discusses two of them. Both are forms of what may be termed effect amplification. It is known for some time from cytogenetic research that the number of germline mutations caused by exposure to

ionising radiation can increase during successive generations. More recent data suggest that, besides this ‘amplification in time’, there may exist an ‘amplification in space’. Genetic studies of animal populations show that acute doses of gamma-radiation cause a large increase in so-called minisatellite mutation rates, much larger than can be explained by the number of energy loss events involved (minisatellite loci are repeated units of short DNA fragments). The mechanism of this high sensitivity is not known at present. It may be that radiation first triggers instability of the genome, which then operates on the hypersensitive locus to change the repeat number. According to some researchers, minisatellite mutation rates are also unusually high in exposed populations after the Chernobyl accident. Dr Bridges thinks the human data are still unclear, because of various methodological problems with the analysis. Nevertheless, such biomarkers and analytic techniques promise new insights into the way in which radiation interacts with living organisms. Apart from this, the relevance of these phenomena to human health is a matter of debate. The same holds for the adaptive response. Such effects should at least make one cautious with respect to modifcations of dose-effect relationships and regulatory decisions.

Discussion

In their introductions the speakers stated that new studies add interesting dimensions to the understanding of the actions of ionising radiation, but that the present evidence does not justify a readjustment of the conceptual framework for risk assessment. The participants at the conference agree. But they conclude as well that evidence like this should always be carefully evaluated in choosing risk models for radiation protection purposes, also if the judgement is that the findings cannot be quantitatively taken into account.

It is argued that only (multidisciplinary groups of) experts are in a position to decide when and how to adjust the framework of analysis on the basis of such mechanistic evidence. To this end, precise analytical tools should be developed for comparing and coupling experimental and human data. Usually researchers will first attempt to unravel events, and their dose dependencies, in experimental systems (the easier task). Next they should examine to which degree this information corresponds with epidemiological data. Agreement between mechanistic data and the broad predictions from epidemiology may then allow more confident judgements on cancer risks at low doses.

Session III UV radiation

Introduction (Van der Leun)

From a risk assessment perspective UV radiation differs from ionising radiation in a number of respects. Firstly, the range of doses to which one may be exposed lies approximately within only one order of magnitude: outdoor workers in the Netherlands receive a mean of 300 MED per year (MED: Minimal Erythema Dose, the dose causing a just visible reddening in the average white skin), indoor workers about 100 MED per year. The maximum UV dose from sunlight in the Netherlands is about 2000 MED per year. Secondly, for a sufficient production of vitamin D3 in the skin, about 50 MED per year is required. So beneficial effects of UV exposure have to be considered as well. Thirdly, problems of extrapolation appear to be smaller: over the range of 10 to 300 MED a clear dose-response has been observed for some types of skin cancer.

Cellular effects and repair mechanisms (Mullenders)

Dr Mullenders follows with a brief presentation on aspects of DNA damage and repair. UV-B radiation (with relatively short wavelengths) induces predominantly direct lesions in DNA, such as pyrimidine dimers, whereas exposure to UV-A radiation (with longer wavelengths) enhances indirect oxidative damage to DNA bases. Considerable progress has been made in understanding the repair systems organisms have developed for coping with these forms of damage. Principal defence mechanisms are base excision repair and nucleotide excision repair. Dr Mullenders pays special attention to the latter, which has two different pathways. Transcription coupled repair only takes place in actively transcribed DNA and it seems to occur to a comparable extent in mice and men. Experimental research suggests that the mechanism depends on dose, low doses inducing a relatively better repair than high doses. A second pathway operates for genomic regions that are non-coding. This form of repair is higher in men than in mice. Dr Mullenders mentions a number of topics for further investigation: the connections between deficient DNA repair, genomic instability, and cancer risk; the nature of the relationship between decreased DNA repair and enhanced apoptosis; the influence of dose on various cellular processes; and the comparability of data on mouse and man.

Intercellular effects and repair mechanisms (Ullrich)

There are three intervention points in which protective mechanisms stop the cascade of steps leading from UV exposure to skin cancer: melanogenesis (the production of melanin, which shields the skin from additional UV damage), DNA repair (the theme of the previous introduction), and immune surveillance. Dr Ullrich presents some data on the effects of UV radiation on the skin immune system. He discusses in particular the apparent relation between DNA damage and immunosuppression. Recent experimental findings suggest which pathways may be involved. DNA damage caused by UV radiation induces the release of cytokines, which act to stimulate carcinogenesis by blocking immune surveillance. Unrepaired pyrimidine dimers could be the trigger of shift in immune response from an active to a suppressive mode. Moreover, because cytokines mediate communication between cells, DNA damage in one cell can alter gene expression in undamaged cells. When analysing these phenomena and hypotheses, it is important to note some limitations and uncertainties. For example, the effects in question have not yet been studied in man. Neither is it clear how they depend on dose. In Dr Ullrich's opinion, the relation will probably turn out to be non-linear.

Combining epidemiological and mechanistic information (De Gruijl)

Dr De Gruijl addresses partly the same issues as Drs Mullenders and Ullrich. Moreover, he touches upon the comparison of data on experimental photocarcinogenesis with results of epidemiological investigations. As to the latter, there is a clear dependence of skin cancer incidence rates on geographical latitude, pointing to an influence of UV radiation. With some adjustments the dose-time-response relation for mice can be fitted to human data. The model obeys Weibull statistics. Lack of data on tumor progression precludes using biologically based models at the moment. Dr De Gruijl also considers the perspectives of molecular epidemiology, which investigates associations between certain molecular or cellular changes and risk of disease. Such studies are only promising when insights into pathogenetic processes are substantial. Researchers have identified particular mutations in the p53 tumor suppressor gene that appear to be related to UV exposure, and that are consistently found to be frequent in human skin tumor cells. However, the p53 pathway, which is likely to be important to tumor progression, may become dysfunctional through other alterations as well. So caution is warranted in using p53 mutations as biomarkers. Still, increasing understanding of UV carcinogenesis might provide a set of relevant biomarkers, e.g. with respect to individual susceptibility.

Discussion

The major topic of discussion during this session is the importance of mechanistic modelling to risk assessment. The cellular and intercellular response to UV exposure has been studied relatively well and the processes involved have been described in considerable detail. In fact, various questions concerning mechanistic aspects can at least be partially answered. Yet the participants do not quite agree on the urgency of developing mechanistic models for UV radiation. Some argue that one should always use all available data in construing risk models. Others feel that, for assessing and managing risks, the marginal returns of — continuously — incorporating mechanistic data in exposure effect models will be small when there is a reasonably large set of phenomenological data on the effect of low level exposure, as is the case for UV radiation. Statistical models may then suffice.

On the other hand, there appear to be no substantial differences of opinion about research questions relating to cellular events and their possible interactions. Dose dependencies are a major issue. In addition most participants advocate the development of increasingly sophisticated mouse models to help clarify the links between various levels of biological organization, to pinpoint variabilities in susceptibility, and to identify similarities and differences between mice and men.

Session IV Dioxins

Introduction (Neumann)

The term ‘dioxins’ stands for the large group of polychlorinated dibenzo-p-dioxins, the most toxic of which is TCDD. A great deal of research has been done on the many adverse effects of this agent and on its modes of action. Recently IARC issued a 700 page report which presents an overview of the experimental and human data. Mainly on the basis of mechanistic insights TCDD has been classified as a so-called class 1 carcinogen (proven carcinogenic in humans). The other dioxins have been put in class 3 (not classifiable as to its carcinogenicity in humans) due to a lack of data. Dr Neumann brings up two topics for discussion. The first concerns the discrepancy between risk evaluations of different organizations. For reasons of scientific and administrative transparency it is important to pinpoint where different courses are open to risk analysts. Among other things, choices have to be made regarding the ranking of endpoints and the methods of extrapolation. Secondly, the IARC classification system might provide clues for grading evidence with respect to risk modelling decisions.

Using in vitro and in vivo data on carcinogenicity (Van den Berg)

Dr Van den Berg, who was a member of the IARC working group on dioxins, focuses on the arguments resulting in a class 1 assignment to TCDD. Experimental research shows that this chemical is a multisite and transspecies carcinogen. Tumors are found primarily in skin, liver, and lungs. Epidemiological investigations among highly exposed workers (including an IARC multicountry study) confirm these experimental findings to a considerable extent. In the Seveso cohort study, however, different tumors have been observed. The difference might be due to latency period, exposure circumstances, and the possible influence of other agents. The mechanism of action has been extensively discussed in the IARC working group. Various studies suggest that the so-called Ah receptor is involved in the process of carcinogenesis. In this connection particular hormones may play an important role as well. Currently a number of ideas about relevant mechanisms are being explored. In summary, IARC classified TCDD as a class 1 carcinogen on the following grounds. High exposure increases overall cancer mortality rates. TCDD is a multisite carcinogen both in experimental animals and in humans. In addition toxicokinetic evidence points to parallel cellular processes, at high exposure levels, in experimental animals and humans: IARC members agreed that the Ah receptor has similar functions in these species. However, the functions seem not to be fully identical, because quantitative differences have also been observed. According to IARC, questions about the shape of the exposure effect curves at low levels of exposure can presently not be answered with any confidence. Although TCDD is considered not to be genotoxic, it is not clear whether a threshold or a non-threshold model is more appropriate for risk assessment. Other endpoints, such as developmental and reproductive effects, should then be carefully studied as well, in particular because they might be more sensitive than cancer incidence or mortality rates.

Using in vitro and in vivo data on developmental effects

Combining epidemiological and mechanistic information (Silbergeld)

Put simply, molecular epidemiology may make the ‘black box’ between exposure and disease more transparent. Many scholars assume that events measured at the molecular level are relevant to and predictive of events in more complex systems, like human beings. Dr Silbergeld believes that these new epidemiological techniques may be particularly useful in studies of low dose effects. Firstly, the events associated with low exposure to environmental stressors are likely to be best observed at the cellular level.

Secondly, the increased precision of biochemical measures allows for more sensitive detection of effects. Before addressing the latest insights into the biological and health effects of low level exposure to dioxins, Dr Silbergeld outlines the main results, opportunities, and limitations of this field of research. Molecular epidemiology has been of greatest assistance in refining exposure measurement. In fact the measurement of toxicants in blood or other compartments has in quite a few cases been the standard for defining exposure for several decades. More recent developments relate to the identification of markers which are intermediate between exposure and preclinical pathophysiology. The best described, and still most frequently utilized, set of such markers concerns carcinogens and cancer risk. Sensitive methods have been developed to detect interactions of chemicals with DNA or proteins. However, the interpretation of DNA or protein adducts and their relevance to risk assessment completely depend on the quality of the pathogenetic understanding, which is often still in its infancy. Molecular epidemiology has also been used to define effects more precisely and to examine host factors that modulate the relationships between exposure and effect. For example, analysis of the types and locations of p53 mutations might become increasingly important to the study of chemical carcinogenesis. The identification of so-called 'susceptibility genes' is another major area of research. Although susceptibility may involve many events other than genotype, genetic differences within populations are likely to be informative when one attempts to explain the variability in human response to exposures.

What have molecular biology and molecular epidemiology to offer to analysts assessing the risks of dioxins? In Dr Silbergeld's opinion, notwithstanding the large literature on the mechanistic toxicology of dioxins, the gap between the increasing knowledge of the early mechanistic events and the major toxic manifestations of dioxin exposure (reproductive dysfunction, birth defects, cancer, immune suppression) remains large, and the usefulness of molecular tools to the epidemiologist unclear. Yet, some links of the exposure effect chain are reasonably well understood. The highly toxic dioxins and related chemicals, especially the PCBs, act in a manner similar to hormones, by binding to the Ah receptor. This receptor appears to affect the transcription of particular genes, such as the estrogen receptor, keratins, and growth factors. At present it is not clear that there are any exposure markers (e.g. induction of CYP450 enzymes) more informative than direct measurements of dioxins in human serum and adipose tissue. Neither have susceptibility markers as yet been clearly identified, despite the existence of substantial species differences. Since dioxins are not appreciably metabolized, it is not likely that genotypic variations in metabolizing enzymes play an important role. Recent data suggest that differences in response may be due to variations in the so-called Ah receptor nuclear translocator protein. It is possible that some of the target genes for dioxin action through the Ah receptor are

polymorphic. Dr Silbergeld concludes her contribution by considering early outcome markers, which most interest risk analysts, especially because many of the low dose effects of dioxin probably increase the risks of chronic diseases. Enzyme induction is a relatively sensitive response, but it is highly variable among individuals and is not specific to dioxins. Work done by various research groups indicates that changes in growth factor pathways might predict later events associated with both developmental effects and cancer. However, these changes mainly occur in tissues which are not accessible to the epidemiologist. Dioxins also have a range of effects on the immune system. But in view of the complexity of the events and given the unclear relationship between immunotoxicity and health effects of interest, such as cancer or reproductive dysfunction, immunologic markers can currently not be used as predictors of disease. Communication between toxicologists and epidemiologists is indispensable for elucidating those processes that are most needed in opening the 'black box' between exposure and disease.

Discussion

One reason why dioxins were selected as a case for this conference is that, in contrast with ionising radiation and UV radiation, they are not only carcinogenic but also have a range of other biological and health effects at low levels of exposure. Cancer risk was until recently the main variable for which exposure effect models, including mechanistic ones, have been developed. The study of other endpoints has often been limited to establishing so-called no observed adverse effect levels. The participants argue that this classical dichotomy, which has frequently been supposed to correspond in broad outline with the difference between non-threshold and threshold effects, is getting obsolete. Progress in the understanding of mechanisms seems to call for more refined systems of classification and more detailed principles of description.

Techniques used in mechanistic cancer risk modelling might contribute to determining quantitative exposure effect relationships for sensitive endpoints such as developmental and reproductive effects. Some participants point out that the scientific literature contains a wealth of data on basic cellular processes, e.g. on how cell cycles are controlled. Yet initiatives to design models which interpret the available data for purposes of risk assessment have so far been remarkably scarce.

A major problem is to identify valid, sensitive measures (biomarkers) which are predictive of clearly adverse effects or disease outcomes. For it is possible that some biological effects are nothing more than normal physiological adaptive responses. However, according to a number of experts even such effects might sometimes be relevant to risk assessment, because of potential variations in physiological resiliency between individuals.

Session V Scientific possibilities and limitations

In this session general issues in risk modelling and in the biology of low level exposures are addressed. Comments and discussion have been moved up to the concluding session of the working conference.

Remark on classifying carcinogens (Neumann)

Continuing his introductory remarks on categorizing evidence in the previous session, Dr Neumann outlines a new classification of carcinogens in Germany. The German MAK-Kommission, which proposes health-based occupational exposure limits, recently drew up a new scheme. It consists of five groups, the first three corresponding to those of the EU: (1) substances carcinogenic to humans; (2) substances carcinogenic in experimental animals; (3) substances suspected to be carcinogenic; (4) substances with carcinogenic potential for which genotoxicity plays no or at most a minor role. No significant contribution to human cancer risk is expected, provided that the MAK value is observed. (5) substances with carcinogenic and genotoxic potential, the potency of which is considered to be so low that, provided the MAK value is observed, no significant contribution to human cancer risk is to be expected. Regulation of chemicals in categories (4) and (5) will thus be based on mechanistic information — nongenotoxic versus genotoxic — and the possibility to assess the carcinogenic potency at low doses.

Synthesis with a view to modelling (Portier)

New techniques, new methods, and new data emerge constantly, but it is not always clear how they can be useful to risk assessment: there is no simple arrow going from science to policy. Having said this, Dr Portier notes that usually only part of the information on toxicity is incorporated in risk models. However, toxicological evaluations of chemical agents should no longer be simply based on outcomes of bioassays or epidemiological studies. There is a, sometimes considerable, increase in information on the effects of an agent on processes like signal transduction, gene expression, endocrine signalling, cellular proliferation, and DNA interactions. Dr Portier is a champion of an integrative, yet at the same time pragmatic approach: one should develop a variety of models and test them against all available data. If a particular model makes sense in terms of these data, it can be used in risk assessment. At the US National Toxicology Program methods of experimentation and analysis are developed to strengthen the scientific foundation of risk evaluations. This includes

advancing collaboration between researchers with different professional backgrounds. Dr Portier elucidates his position by presenting exposure effect models for TCDD. One model attempts to integrate all experimental animal data. It describes the kinetics and dynamics of TCDD in rats and it encompasses about 100 equations and 200 parameters. A similar model for humans could not be developed due to a lack of data. Less sophisticated approaches using statistical models generally involve much larger uncertainties with regard to extrapolation procedures. They are especially useful for assessing risks in populations exposed to levels approximately within the range of observations. When mechanistic data are few, these models may be the best we have. Dr Portier uses a formula with a shape parameter to evaluate, for a large number of endpoints, whether the available data on TCDD are consistent with threshold or non-threshold exposure effect curves. About fifty percent of the endpoints fits a threshold model and about fifty percent a non-threshold model. Weighing the relevance to health of the endpoints under analysis and combining the corresponding data may then be the best approach for risk assessment. It might at least shift the edge of extrapolation downwards.

Significance of cellular and intercellular processes (Trosko)

Dr Trosko emphasizes that there are more things in a cell than DNA, and that carcinogenesis involves more than mutagenesis. It is important to take the evolutionary context into account. During evolution multicellular organisms survived by adaptive responses to both endogenous oxidative metabolism and exogenous chemicals and low level radiation. The defence repertoire exists at all levels of the biological hierarchy. Roughly speaking, three levels of communication can be distinguished: extracellular (‘large distance’) signalling (e.g. hormone action), intercellular (‘short distance’) signalling, and intracellular signalling. Dr Trosko pays special attention to intercellular events. He contends that so-called gap junctional intercellular communication is of crucial importance to many fundamental biological processes, from early embryogenesis to regulation of cell growth later in life. Modulation of gap junctional communication, by the action of e.g. cytokines and growth factors, is likely to play a significant role in the process of carcinogenesis: various studies indicate that blockage of these communication channels may act as an endogenous tumor promoter. Conversely, it is possible that intercellular signalling mechanisms provide protection of any cell hit by e.g. a radiation track through the sharing of reductants and by triggering apoptosis. Dr Trosko has developed a tissue culture system in which the effects of low doses on gap junction intercellular communication can be examined. These experiments may help predict the effects of low level exposures on complex organisms.

Session VI Perspectives for risk assessment: final discussion and recommendations

The debate focuses on possibilities to make more effective links between science and policy. As to the scientific side of that relationship, the participants critically review promising approaches and note a number of issues in need of clarification. Opening the black box between exposure to a particular agent and its health effects is seen as the major route to progress. Various types of research can shed light on various parts of such a black box, generating a diversity of biomarkers. It is argued that comprehension of low level effects will evolve iteratively from application of a variety of biomarker variables relating to different levels of biological organization. At present, only a few markers are available that can, for instance, be effectively used in epidemiological studies, but applications will no doubt increase. Future usefulness of biomarkers strongly depends on the rate at which problems with respect to their validity, reliability, and generalizability will be overcome. The participants endorse the general paradigm of human-animal parallellism. Systematically comparing animal and human data, ranging from results of in vitro methods to outcomes of in vivo approaches, is critical for determining fundamental links of exposure-effect chains and for identifying uncertainties with regard to interspecies extrapolation and exposure-effect modelling. Some participants have high expectations of the development and use of transgenic mice, which can be tailored to study the influence of particular molecular events on physiological variables. This includes the examination of gene environment interactions and of variations in susceptibility. However, it is remarked that some endpoints, e.g. neoplastic lesions, may lend themselves more to comparison than others, e.g. particular neuropsychological phenomena. When it comes to formulating guidelines to create a framework for risk modelling, the participants recommend to be pragmatic and open minded: a variety of models can be useful for risk assessment. In the final analysis, experts should decide on an ad hoc, case-by-case basis. Consensus emerges that analysts should keep themselves informed about technical advances in risk assessment methodology. Researchers have been developing new modelling techniques which attempt to utilize more of the available scientific knowledge and expertise. In this connection it is essential that scientists with different backgrounds collaborate. In fact the risk assessment enterprise can be structured as a modular activity: when the experts feel that the evidence concerning particular processes is solid enough, models can be tied to it. During the conference Dr De Vries Robbé showed how communication and collaboration between professionals can be promoted by using so-called cognitive maps, which attempt to make explicit the ideas and conceptual frameworks taking root in various biological subdisciplines.

Quite a different matter is whether sophisticated modelling activities are worthwhile from the perspective of risk management. Many participants emphasize that the problems under analysis should always be put in a societal context. This entails examining actual options for reducing exposures, evaluating the costs and benefits involved, and performing sensitivity analyses with regard to the modelling of risk as a function of exposure. Parties who are affected by the risk management problem should help frame the questions for risk assessment, e.g. which endpoints should be considered. Basically, they should determine how high the stakes are and how deep the analysis should be, knowing that it is not practical to crack a nut with a sledgehammer. Other participants add that different perspectives can come up, which may be referred to as 'agent-orientated' and 'health-orientated'. The former point of view more or less coincides with prevailing or legally required methods of risk assessment, whereas the latter addresses the usually multifactorial nature of health problems and tries to determine the influence of exposure to one particular agent against the 'background' of many other contributing risk factors. It is concluded that techniques should be developed for analysing the risks of combined exposures and for establishing the contribution of individual stressors. Some participants state that sophisticated modelling will become the easier and less time consuming, the more the experience with handling the analytical instruments increases. As a matter of fact it can be expected that many basic modelling components apply to a large number of agents: the wheel does not have to be reinvented time and again. Whether the application of these modelling techniques should be accompanied by statements detailing the degree of evidence, is a topic that, according to the participants at this conference, may warrant another workshop.

Some afterthoughts

Given the diverse set of participants it is noteworthy that areas of agreement were large. The participants appeared to express similar views on many recurrent themes:

- Although hormesis cannot be excluded on theoretical grounds, there is at present no hard evidence for it.
- It is true that insights into molecular and cellular effects of exposure to physical and chemical agents are sometimes rapidly increasing, but many questions remain to be answered. It is generally poorly understood how exposure timing and exposure dose could influence the potential cellular effects, such as 'no change', mutations, cell death by necrosis or apoptosis, or altered gene expression. Furthermore, there is usually insufficient scientific knowledge to establish precisely how each of these potential effects at the cellular level could contribute to

various physiological or disease outcomes, such as cancer, developmental effects, or reproductive dysfunctions.

- Progress can be made by systematically comparing mechanistic and phenomenological information on the one hand, and animal and human data on the other hand. However, there are no clear cookery-book procedures for combining data with respect to low level risk assessment: in the final analysis, experts should decide on a case-by-case basis.

The conference left open the question of whether it is always appropriate to use sophisticated modelling techniques. Many participants felt that such techniques should only be used when the societal stakes are high. Others believed that practice makes perfect.

Annex **D**

The debate about low levels of exposure

Eert Schoten

This note concerns scientific research into the effect on the human body of low doses of physical and chemical agents, and the significance of various research data for health assessments of such exposures. It is an extremely general exploration of an issue that will be the subject of a working conference to be held next year, under the auspices of the Health Council of the Netherlands, and is intended to serve as background information for working out the details of the conference programme.

1 The background to the problem

Our insight into the effect of radiation and chemical substances on health is less than we would wish. We know that exposure to high doses of these agents can damage organs but, leaving aside the case of accidents, there are questions about their potential harmfulness at the relatively low exposure levels that occur in the physical or working environment. Do high and low levels of exposure only differ in the strength or frequency of what are, for the rest, similar effects, or are there more likely to be essential differences in the reactions? If there are, what should be considered as ‘high’ and ‘low’? It is not possible to get around these questions when standardizing exposure levels to protect or promote the health of the public.

The Health Council has a long tradition of assessing scientific data that can be used to support the environmental and occupational exposure limits in question. A thread that runs through the recommendations of the Council is the fact that, if available, epidemiological data on the effect of low levels of exposure usually reveal very little. In general, it is impossible to exclude any particular negative effect, such as additional harm of any description to the people exposed vis-à-vis people in the control group, or the reverse of this, i.e., any positive effect, or the absence of any effect at all.

It is therefore necessary to resort to other sources of information to express an opinion on the possible health consequences of exposure conditions of this kind. A whole range of data can then qualify for consideration, such as the results of epidemiological studies into the effect of high levels of exposure, the outcome of animal tests (usually also at high doses), and information about the way in which the molecules of an agent interact with those of a cell.

But these indirect approaches are not without problems. Because of their indirect character, we cannot avoid specifying how data of this kind can shed light on the effect of low doses in the human body. Each of the sources of information referred to presents us with just as many extrapolation problems in terms of what ‘high’ implies for ‘low’ (the question already raised in the introductory paragraph), what an ‘animal’ can reveal about ‘human beings’ and what a ‘molecule’ can tell us about an ‘organ’? Answers to these questions result in recommendations to use particular extrapolation models. Using these, it is possible to deduce what effects can be theoretically expected for low levels of exposure, even if the effects are not demonstrated directly as a manifestation of disease symptoms, at the level of organs.

2 Discussion of models

The models that have been used or proposed have been a point of discussion right from the start. Besides covering the scientific aspects (How strong is the empirical evidence for certain hypotheses that form the basis of the models? How can these hypotheses be tested and further specified?), the debate is also concerned with questions that have a normative or, put another way, political tint (How should the uncertainties be dealt with? Are simplifications necessary from the administrative point of view?). In recent years, the discussion seems to have been getting more heated. This is partly because of the rapidly advancing developments in cell and molecular biology, and partly because of a growing and more frequently expressed scepticism about the reasonableness of various standard setting procedures. Many people are asking themselves whether the balance is right between the costs of all kinds of laws and rules, on the one hand, and, on the other, the supposed benefits, viz. the prevention or reduction of damage to health? The question from the scientific point of view is whether the opinion about the

damage caused by exposure to low doses can be maintained, in the light of the most recent insights into the biochemical machinery that cells have for adapting themselves to stimulation from outside.

This issue is also frequently discussed by the Health Council. Some time ago, the Standing Committee on Radiation Protection discussed the question of whether there is a possibility that cellular defence mechanisms induced by exposure to background radiation (ionising radiation that occurs naturally) might actually indirectly reduce the risk of cancer (see section 5). This is contrary to the current standpoint on radiation protection which is that background radiation increases the risk of cancer. The discussion gave the former chairperson of the Health Council cause to have a further exchange of ideas with a number of council members about this possible 'effect compensation' and, more in general, about the significance of data on the mode of action, in terms of estimating the risks of exposure to low doses. Besides the Council's chairperson, Drs Blok, Feron, and Lohman took part in the discussions. Below, the comments of the aforementioned council members are placed in the context of a number of scientific and social trends.

3 The message of the critics

When articles appear in scientific literature under titles such as 'The triumph of theology over science: the non-threshold effects model' (Sag94) and 'Cancer risk assessment: the science that is not' (Gor92), it is obvious that we are dealing with a topic that will continue to be a point of discussion for some time to come. These authors believe that making assessments of the risks of exposure to low doses of carcinogenic agents has a lot in common with making a declaration of faith. According to them, these agents only appear to be carcinogenic at high doses, and then often only in animals. Simply assuming that exposure to low doses increases the risk of cancer, albeit to a relatively limited degree, fails to pay sufficient heed to indications that, in reality, matters are a lot more involved. In particular, the rigorous application of the linear no-threshold hypothesis should be blamed for this. It is a dual hypothesis: (1) even the lowest doses of these agents ('absorbed dose' in the case of ionising radiation, and 'ingested amount per unit of body weight' in the case of substances) can cause irreversible damage to cells and thereby increase the risk of cancer; and (2) in the case of low levels of exposure, the risk increases proportionately with the dose. Within the scope of this, the qualification 'low' is usually simply described as 'not high'. High doses are those that result in acute organ damage.

The aforementioned critics and their supporters object to this position. Most experts now think that carcinogenic agents cannot all be placed in the same category. Meanwhile, the opinion is fairly widespread that there are so-called genotoxic and

non-genotoxic agents. According to this opinion, the first category is capable of damaging cell nuclei permanently, the second appears to particularly affect cell division and cell proliferation, often by means of a reversible process. The implication of this is that the harmful effect in the second case does only occur above a certain threshold dose, whereas in the first case it does not. Health Council committees have also subscribed to this view (GR78, GR94).

However, this differentiation is not the main concern of the critics, although they appreciate the focus on the way in which agents affect cells. In their view, it is more important that the ability of agents to damage DNA molecules in cell nuclei or to affect cell division is not the whole story, as other processes that can interfere with those mentioned above also occur in cells. If the developments in molecular biology and cell biology have taught us anything, it is that a complex combination of actions takes place with numerous possible reactions. We are learning increasingly more about the cascades of biochemical reactions in and between cells, both in healthy tissue and in tumours (Kar95, Spo96, Var93). For example, it has been known for some time that the p53 tumour-suppressor gene can protect DNA molecules by temporarily blocking cell division after damage, thereby enabling repair mechanisms to do their work. Recently there have also been some indications that p53 itself can trigger certain repair mechanisms (Mar94). This information increases the insight into what happens in cells after they receive an external stimulus, for example. However, this also presents new questions about the precise relationship between the various subprocesses and the results of their interaction under different conditions. There is still no clear answer to these questions.

4 The BELLE initiative

It is still too early to make any definite statements but, in terms of estimating and assessing risks, the interest in the importance of new insights is growing. An example of this is the initiative in the United States in the early nineteen nineties, known by the acronym BELLE: Biological Effects of Low Level Exposures. This group of researchers set themselves the task of examining more systematically the various processes that occur in cells and organs when they are ‘hit’ by radiation or chemical substances. Two symposium collections have been published thus far under the auspices of BELLE (Cal91, Cal94). They also publish a regular newsletter.

The articles in the collections and the newsletter are quite varied, with reports on observational studies and theoretical considerations appearing alongside each other, together with toxicological and epidemiological discussions and contributions on carcinogenic and non-carcinogenic agents. However, they have in common that they

are often concerned with dose-response and dose-effect relationships that are in some way different from what is currently taken to be the case. As already mentioned briefly, in many circles it has become the established view that there is a fundamental difference between genotoxic and non-genotoxic agents. The harmful effect of the former is considered to be characterized by dose-response relationships without a threshold. (There are differences of opinion about the precise shape of the relevant curves, whether they are linear or non-linear for example.) The second category, regardless of whether they are carcinogens, are supposed to only result in a harmful change above a certain threshold dose, below which nothing happens. This is a clear picture, which organizations that have to set standards can work with easily. The BELLE group is illuminating the picture's cracks and unevenness: genotoxic agents for which there are nevertheless indications of a threshold, and also various agents that first have a favourable (or at least not unfavourable) effect as exposure increases, but then later show an unfavourable effect.

If we assume that these findings cannot generally be ascribed to incorrect research techniques, but that they indicate processes that may well be difficult to reveal but that nevertheless exist, there do, indeed, appear to be reasons for examining the aforementioned standard picture more critically than in the past. However, the question concerns how that standard picture may need to be changed, especially bearing in mind standard setting procedures. It is one thing to point to the multitude of favourable, unfavourable or neutral processes that occur in and between cells, but it is a completely different matter to describe those processes quantitatively as a function of the dose, both separately and in their interactions.

5 The possibility of effect compensation

The exchange of ideas between the council members mentioned in section 2 was mainly concerned with the fundamental scientific preconditions in this whole area: are 'different' dose-response or dose-effect relationships possible according to present physical and biochemical insights? In concrete terms, the discussion focused on the effect of low doses of ionising radiation and the possibility of 'effect compensation'. The passage of ionising radiation through cells involves the release of energy packets that are considerably larger than those exchanged between molecules during the normal functioning of cells. This can result in what appears to be unfavourable damage to the DNA in the cell nucleus. However, it can also result in extra biochemical repair reactions that can partially undo the harmful effects of other, possibly stronger, genotoxic agents, to which such a cell is also exposed (see section 6.1). It is therefore conceivable that the net result of all these interactions could be positive for a given

dose range; in other words, the risk of developing cancer can, on balance, be reduced (effect compensation).

On the basis of a rough calculation, council member Blok explained that for an absorbed dose of 10 mGy per year — a little more than that resulting from background radiation — a random body cell would be hit about once every two days and a random cell nucleus would be hit about once every quarter. According to him, it is possible to conclude from this that any effect compensation is more likely to be caused by the induction of protection mechanisms in the cytoplasm than in the cell nucleus. In the latter case, any such mechanisms would in fact have to remain operational for several months after the cell had been affected. The effect of chemical genotoxic agents can also be looked at from two points of view: the damage caused to cell nuclei following exposure and the defence mechanisms that are mobilized. The interactions mentioned and their dependence on the dose may be different for each agent. The present level of knowledge precludes the possibility of making any more detailed statements about this, let alone formulating any particular laws.

The lack of insight is also clear from the language used in scientific literature on the subject. It is often full of metaphors: researchers talk about ‘stimuli and reins’ or about ‘invaders and defenders’, complete with ‘rules’ according to which the battle between both proceeds. This is all based on the idea that a system of balance (homeostasis) exists inside and between cells, which can take an occasional blow. However, as indicated, it is difficult to provide any quantitative substantiation of these processes, or they have to involve simulations. In this regard, if one takes into account the mobilization of the repair mechanisms or the increase in cell death, both of which are assumed to be saturable, model calculations are instructive in showing how the risk of cancer first decreases and then increases as a function of the dose, after exposure to a fictitious genotoxic agent (Ste94).

6 Trends in scientific research

However, calculation exercises of this kind are of little use if they are not properly related to empirical data. Unfortunately, in all scientific advances, the picture of the biochemical machinery in and between cells is still very diffuse and fragmented. However, work continues to reduce the knowledge gaps in the various, partially overlapping, fields of research. These fields include the following.

The research into DNA repair

Various mechanisms in the cell nucleus help maintain the DNA structure by, as far as possible, removing defects that occur during replication and damage that results from exposure to stimuli in the environment. There is a whole range of repair processes. Some involve a single step, whereas others involve a series of steps. Some focus on the entire genome but others are concerned with specific genes in the genome (Boh95, Cle94, Han95). The last few years in particular have produced some rapid developments in techniques for tracing and further delineating repair mechanisms in genes.

The discovery of what are termed nuclear factors in the cytoplasm of mammalian cells was also of major importance. These are proteins that are activated by extra-cellular signals. They subsequently penetrate the cell nucleus as a transcription factor, where they cause expression of particular genes, so that certain defence proteins are produced. An example of this is the nuclear factor NF-kB (Sch92, Tha95). Many stimuli (viruses, bacteria, cytokines (intercellular signalling molecules), UV radiation, ionizing radiation and certain chemical agents) appear to activate this factor (and related factors). The number of target genes in the cell nucleus appears to be even larger. However, still little is known about the function of the defence proteins that are produced. They include cytokines, which are able to produce a high state of alert in neighbouring cells that may not have been affected. A possible effect compensation would therefore be able to spread like ripples in a pool.

The research into oxidants and anti-oxidants

However, a real battle takes place before mechanisms that repair damaged DNA can become active. The battle is between, amongst other things, what are referred to as oxidants and anti-oxidants. Oxidants are substances that can damage all kinds of macromolecules, including DNA, through an oxidation process. This involves reactive interim products (radicals) in the reduction of oxygen to water. They occur in cells after penetration by, for example, ionising radiation, but also especially as a result of normal metabolic processes. Anti-oxidants impede or delay these oxidation reactions in biomolecules. Some examples of oxidation inhibitors are vitamin E, vitamin C and carotenoids, which occur in vegetables and fruit. Anti-oxidants do not offer any generic protection against oxidation reactions; the effectiveness of the protection is highly dependent on the nature of the threat (the radical type), the structure of the molecules under threat and the mechanism by which the anti-oxidant works. (Are radicals blocked? Is their formation impeded? Is the damage repaired?) (Hal95).

Both issues, oxidative stress and defence possibilities, are receiving increasing attention in the search for the causes and pathogenesis of degenerative diseases, such as cancer, cardiovascular diseases and diseases of the nervous system and the immune system (Ame93, Ame95, Bor93, Shi94). The interesting thing about this development is that attempts are being made to consider cellular processes in cohesion and to place them in perspective. A couple of salient points (Ame93, Ame95) within the scope of this are: the oxidative damage caused by exposure to synthetic chemicals and radiation is very much less than that which results from the normal metabolism in cells; consequently, a reduction or deferment of the damage and the associated degenerative diseases may be more readily attained through a reduction of the metabolic rate (eating less on a daily basis) and by following a diet that is rich in anti-oxidants (a lot of vegetables and fruit), rather than through extremely stringent environmental protection standards.

Some people are calling for anti-oxidizing vitamins to be added to foods or for these substances to be taken in addition to the normal diet. According to the Health Council's last annual report, at present the health benefits of this have not been sufficiently documented (GR95). The Council will soon be looking at this issue in more detail (GR96).

The research into toxicokinetic and toxicodynamic processes

Traditionally, in studying the consequences of exposure to chemicals, a distinction has been made between the toxicokinetic phase, which is concerned with the absorption, distribution, biotransformation and excretion of a substance, and the toxicodynamic phase, in which the interaction of the molecules of the agent with those in the cells is central. In setting standards, it is important to know which part of the dose (defined as the amount taken per unit of body weight) is 'biologically effective'. Some trends clearly seem set to continue over the coming years (Fre95, Men95). One such trend is the growing interest in PBPK/PD models (PBPK/PD stands for Physiologically Based Pharmacokinetic and Pharmacodynamic). Using these models, researchers want to provide the most explicit and systematic description possible of the aforementioned kinetic and dynamic processes. The predictive power of PBPK/PD models leaves a lot to be desired at the moment; it is not usually possible to deduce any valid dose-effect relationships from them. Their usefulness is more their instrumental value in testing hypotheses. (Why is one organ damaged and another not? How is it that some species of animals are unsusceptible?) The potential carcinogenicity of methylene chloride provides an example of the latter question (Kai96). Exposure to this substance can cause tumours in mice, owing to a metabolic product formed in the nuclei of lung and liver cells, which damages DNA locally. In rats and humans, this metabolic process

occurs outside the cell nucleus, which seems to cancel the carcinogenic potential. Given the present level of knowledge, one of the priorities of the research is to validate PBPK/PD models. Another is the standardization of the methods of determining the many process parameters that are intended to give shape to the models.

A second trend is the study of structure activity relationships (SARs). These relationships indicate how the physical and chemical properties of an agent are connected to its toxicity. The need for SARs mainly arises from the major lack of direct data on the toxic potential of many chemicals. Toxicologists are expected to increasingly attempt to get round this lack of knowledge by using SARs in PBPK/PD models.

The research into biomarkers

Whereas traditional epidemiology concentrates on finding causal connections between exogenous factors and diseases, molecular epidemiology is interested in the links of these cause and effect chains. Exposure and the manifestation of the disease only mark the beginning and end of a continuum of events. In between, all kinds of measurable changes take place, which can provide an insight into the pathogenesis and, in principle, can provide a point of application for prevention or early treatment. Biomarkers is the term researchers use for these changes, as well as for cell structures that are relevant from this point of view. DNA adducts (covalently bonded complexes between DNA and a mutagenic agent) are one example. In molecular epidemiology, a relationship is sought between these biomarkers and diseases, such as the possible link between the number of DNA adducts and the risk of certain types of cancer. Although this type of research is generally considered to hold a lot of promise, opinions tend to differ about how long it will be before useful results are available (Cuz95, McM94, Per96a, WHO95). However, refinements in research techniques are expected to enable the effect of exogenous factors to be better subdivided into the endogenous susceptibility of people, in particular into their genetic characteristics, which can also be considered as biomarkers (Dol96, Per96b, San95, Sch95).

The research into ageing processes

Many of the aforementioned research topics come together in the study of the driving forces behind the origin of age-related diseases and ageing in general. Because (chronic) low exposure to agents is associated with — an acceleration or alternatively a delay in — the onset of degenerative processes of this kind, biogerontology seems to be a branch of science that is especially capable of providing a coherent description of the effect of such exposure. A lively debate is underway at present about the

significance of genetic factors for the ageing process and about their interaction with environmental determinants (Jaz96, Kir92, Lit96, Mar96, Par93, Soh96). The context is usually that of evolution biology: is ageing programmed or does it arise as a trade off between increased chances of reproduction and a shortening of life? Is that trade off optimal or could it be improved? The attractive aspect of the evolution perspective is that it takes into account the time dimension of the effect of exposure to agents, something which has been put forward on several occasions by the Health Council.

7 An information handling problem

As illustrative and limited as the descriptions and examinations given in the preceding sections may be, one thing is clear: there are signs in various branches of science that the method of classification and extrapolation used thus far for estimating the risks associated with exposure to low doses of agents fails to consider some potentially interesting questions. According to the council members, in the light of what is known at present, it is at least possible to say that phenomena such as effect compensation cannot be excluded, even if it is not possible to say much about their plausibility.

The aforementioned signs have not gone unnoticed by politicians, administrators and lobbyists. Particularly but not only in the United States, there are growing objections to the current methods of assessing risks; the estimates, which are associated with uncertainties, are considered to err too much on the safe side. As an extension of this, the procedures for managing risks are under attack; exposure standards are often determined without paying sufficient attention to the costs involved and without regard to the importance of setting priorities (Abe93, Arr96, Mac96, Par95). The call for more attention to be paid to cost-benefit considerations has been heard, insofar as a body like the EPA (Environmental Protection Agency) recently drew up guidelines for methods of risk assessment, in which data on the mode of action of agents have to be considered. This expresses the growing realization that it would be better to consider the uncertainty of the benefits along biological lines, rather than express them in aspecific extrapolation principles (Kai96, Men95, Sto95).

However, for the time being the expectations of many experts are tempered by the lack of insight into all the processes that play a role in the interaction between agents and cells. The council members also believe it will only be possible to say more about the effect of low doses, including what ought to be understood by ‘low’, when the scientific research in the field becomes more systematic and is more advanced. However, an interim solution must be offered, because policy-makers are unable to wait for the results of this. In fact, this concerns an information handling problem (which is incidentally not unique to environmental protection and occupational safety but also applies to ‘evidence-based medicine’ and other complex assessment

processes): how can the various types of data, with all their deficiencies, be ordered (and assessed) so that policy-makers can make optimal use of them in making their decisions?

As far as the Health Council is concerned, this general question should set the course for the working conference. In which case, participants at the conference would have to address problems such as: how can data on the effect and repair mechanisms be incorporated in the dose-effect curve models? Is it possible to make any generic statements about this or are we practically compelled to make agent-specific recommendations? Can we produce assessment methods that flexibly incorporate new scientific insights that lead to appreciable results? What methods do we have available for giving uncertainties a specific place in the risk assessments? Questions like these should be discussed on the basis of a limited number of clear-cut and well-documented case studies.

8 References

Abe93 Abelson PH. Pathological growth of regulations. Science 1993; 260: 1859.

Ame93 Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of ageing. Proc Natl Acad Sci USA 1993; 90: 7915-22.

Ame95 Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proc Natl Acad Sci USA 1995; 92: 5258-65.

Arr96 Arrow KJ, Cropper ML, Eads GC, a.o. Is there a role for benefit-cost analysis in environmental, health, and safety regulation? Science 1996; 272: 221-2.

Boh95 Bohr VA. DNA repair fine structure and its relations to genomic instability. Carcinogenesis 1995; 16: 2885-92.

Bor93 Borek C. Molecular mechanisms in cancer induction and prevention. Environ Health Perspect 1993; 101 (Suppl 3): 237-45.

Cal91 Calabrese EJ (ed). Biological effects of low level exposures to chemicals and radiation. Boca Raton, 1991.

Cal94 Calabrese EJ (ed). Biological effects of low level exposures: dose-response relationships. Boca Raton, 1994.

Cle94 Cleaver JE. It was a good year for DNA repair. Cell 1994; 76: 1-4.

Cuz95 Cuzick J. Molecular epidemiology: carcinogens, DNA adducts, and cancer - still a long way to go. J Natl Cancer Inst 1996; 87: 861-2.

Dol96 Doll R. Nature and nurture: possibilities for cancer control. Carcinogenesis 1996; 17: 177-84.

Fre95 Frederick CB. Summary of panel discussion on the ‘advantages/limitations/uncertainties in the use of physiologically based pharmacokinetic and pharmacodynamic models in hazard identification and risk assessment of toxic substances’. Toxicology Letters 1995; 79: 201-6.

Gor92 Gori GB. Cancer risk assessment; the science that is not. Regul Toxicol Pharmacol 1992; 16: 10-20.

GR78 Gezondheidsraad. Advies inzake de beoordeling van carcinogeniteit van chemische stoffen. Gezondheidsraad. Publikatie nr 1978/19. Rijswijk, 1978.

GR94 Gezondheidsraad. Risk assessment of carcinogenic chemicals in the Netherlands. Regul Toxicol Pharmacol 1994; 19: 14-30.

GR95 Gezondheidsraad. Jaaradvies Gezondheidszorg 1994-1995. Gezondheidsraad. Publikatie nr 1995/13. Den Haag, 1995.

GR96 Gezondheidsraad. Werkprogramma 1996-1997. Gezondheidsraad. Publikatie nr A96/02. Den Haag, 1996.

Hal95 Halliwell B. Antioxidant characterization. Methodology and mechanism. Biochem Pharmacol 1995; 49: 1341-8.

Han95 Hanawalt PC. DNA repair comes of age. Mutat Res 1995; 336: 101-13.

Jaz96 Jazwinski SM. Longevity, genes, and ageing. Science 1996; 273: 54-9.

Kai96 Kaiser J. New data help toxicologists home in on assessing risks. Science 1996; 272: 200.

Kar95 Karp JE, Broder S. Molecular foundations of cancer: new targets for intervention. Nature Medicine 1995; 1: 309-20.

Kir92 Kirkwood TBL. Comparative life spans of species: why do species have the life spans they do? Am J Clin Nutr 1992; 55: 1191S-5S.

Lit96 Lithgow GJ, Kirkwood TBL. Mechanisms and evolution of ageing. Science 1996; 273: 80.

Mac96 Macilwain C. Risk: a suitable case for analysis? Nature 1996; 380: 10-1.

Mar94 Marx J. New link found between p53 and DNA repair. Science 1994; 266: 1321-2.

Mar96 Martin GM, Austad SN, Johnson TE. Genetic analysis of ageing; role of oxidative damage and environmental stresses. Nature Genetics 1996; 13 (mei): 25-34.

McM94 McMichael AJ. Invited commentary - “molecular epidemiology”: new pathway or new travelling companion? Am J Epidemiol 1994; 140: 1-11.

Men95 Menzel DB. Extrapolating the future: research trends in modeling. Toxicology Letters 1995; 79: 299-303.

Par93 Partridge L, Barton NH. Optimality, mutation, and the evolution of ageing. Nature 1993; 362: 305-11.

Per96a Perera FP. Uncovering new clues to cancer risk. Scientific American 1996; 274(5): 40-6.

Per96b Perera FP. Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. J Natl Cancer Inst 1996; 88: 496-509.

Por95 Portney PR, Harrington W. Health-based environmental standards: balancing costs with benefits. Resources 1995 (summer): 7-10.

Sag94 Sagan L. The triumph of theology over science: the non-threshold effects model. Int J of Occup Med and Toxicol 1994; 3: 161-72.

San95 Sankaranarayanan K, Chakraborty R. Cancer predisposition, radiosensitivity and the risk of radiation-induced cancers. I Background. Radiat Res 1995; 143: 121-43.

Sch92 Schreck R, Albermann K, Baeuerle PA. Nuclear Factor kB: an oxidative stress responsive transcription factor of eukaryotic cells (a review). Free Rad Res Comms 1992; 17: 221-37.

Sch95 Schatzkin A, Goldstein A, Freedman LS. What does it mean to be a cancer gene carrier? Problems in establishing causality from the molecular genetics of cancer. J Natl Cancer Inst 1995; 87: 1126-30.

Shi94 Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in ageing. Proc Natl Acad Sci USA 1994; 91: 10771-8.

Soh96 Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and ageing. Science 1996; 273: 59-63.

Spo96 Sporn MB. The war on cancer. Lancet 1996; 347: 1377-81.

Ste94 Stevenson DE, Sielken RL, Bretztaff RS. Challenges to low-dose linearity in carcinogenesis from interactions among mechanistic components as exemplified by the concept of ‘ invaders’ and ‘defenders’. BELLE Newsletter 1994; 3(2): 1-8.

Sto95 Stone R. A molecular approach to cancer risk. Science 1995; 268: 356-7.

Tha95 Thanos D, Maniatis T. NF-kB: a lesson in family values. Cell 1995; 80: 529-32.

Var93 Varmus H, Weinberg RA. Genes and the biology of cancer. New York, 1993.

WHO95 WHO Regional Office for Europe. Guiding principles for the use of biological markers in the assessment of human exposure to environmental factors: an integration approach of epidemiology and toxicology. Report on a WHO consultation. Toxicology 1995; 101: 1-10.

Annex **E**

Note for the conference

1 Introduction

Following recommendations in the background document ‘The debate about low levels of exposure’ (Annex D) the working conference ‘Health Effects of Low Level Exposures: Scientific Developments and Perspectives for Risk Assessment’ will be structured around three cases and three broad questions. These cases are

1 ‘ionising radiation’,
2 ‘UV radiation’, and
3 ‘dioxins’.

The questions are

1 What is the state of knowledge about deleterious effects and defence mechanisms following low levels of exposure?
2 What are the implications of those insights for risk assessment procedures?, and
3 Which types of research should be prioritized to promote evidence-based risk assessment?

Together with the background document this note provides some topical information for the sessions of the conference. It highlights a number of insights, research questions, or controversial issues which play a more or less prominent role in discussions about the impact of low level exposures. Among the recurring themes in the scientific debate are:

- the relative importance of intracellular and intercellular processes,
- the relative importance of adverse and beneficial effects,
- the difference between early and late health effects, and
- the significance of subclinical effects.

The next sections briefly touch upon one or more of these issues. Section 2 provides background information on the three cases of the working conference. Section 3 draws attention to the importance of conceptual frameworks and to the possible contributions from various biological disciplines. The main objective of these sections is to indicate what kind of phenomena and problems the Health Council would like to see addressed by speakers and other participants. To this end subsections 2.4 and 3.2 contain specifications of the three broad questions listed above.

2 Topics in low level risk assessment

2.1 Issues with respect to ionising radiation

- In recent years much discussion has been devoted to so-called adaptive responses: a low priming dose of ionising radiation appears to protect cells from damage of a subsequent high dose. The deleterious effects in question are chromosomal changes and gene mutations (Mut96, NRP95, UNS94). Radiobiologists try to find out what kind of mechanisms may be responsible for these experimental findings. In addition, and more importantly, they are investigating whether similar adaptive responses or protective mechanisms may lead to a reduction in tumour incidence rates following low level exposure. This still remains a controversial issue, with potentially far reaching implications for risk assessment and radiological protection measures.
- In contrast to the adaptive responses, there is recent evidence for at least two mechanisms by which the effects of ionising radiation can be extended beyond the immediate consequences of energy loss events. While there is currently no direct evidence that such dose amplification effects have health implications, the fact that they exist suggests that the implications of the adaptive responses ought not to be considered in isolation in the context of low dose effects.
- Ionising radiation is one of the few environmental stressors for which relatively detailed mechanistic dose-response models have been developed (Bog97, Lue96, Moo90). Models like these give a mathematical description of cellular processes of carcinogenesis, such as (various types of) cell transformation, cell proliferation, cell killing, and cell replacement. Many experts hold that in principle mechanistic

models are better tools for risk assessment than statistical ones. However, so far only the latter have been used for policy purposes.

2.2 Issues with respect to UV radiation

- Quite a lot is known about the way in which UV radiation can induce skin cancer (Gru96). It generates mutagenic DNA photoproducts, leading to dysfunctional genes and malignant transformations, and also downregulates immune responses which can eliminate such transformed cells. Although there exists a whole battery of defence mechanisms, from radical scavengers and repair enzymes to apoptosis and immune surveillance, protection is generally assumed not to be perfect. Yet, with very low daily exposures, a threshold for tumour induction in experimental animals seems to appear, probably because induction times become longer than the lifespan. Another interesting finding is the apparent connection between DNA and cytokines (Yar96). Unrepaired DNA photoproducts cause the release of particular cytokines which stimulate carcinogenesis, whereas repair of DNA lesions checks the release and expression of these cytokines.
- Not only resistance against tumour induction may be affected by exposure to UV radiation, other immune functions are also at risk. Studies indicate that this immune modulation might influence the incidence and severity of allergic, infectious, and autoimmune diseases. However, data necessary to quantitate these risks still seem to be lacking (Sel97).

2.3 Issues with respect to dioxins

- Recent years have seen a growing interest in early subclinical effects of low levels of exposure to dioxin and dioxin-like chemicals (Bir95, Koh96). One of the areas of investigation is the increase in UGT enzymatic activity subsequent to dioxin exposure. This phenomenon is considered to be useful as a biomarker for tumorigenic changes in thyroid hormone levels. So-called physiological dosimetric models are developed to formulate a quantitative dose-effect relationship for this biomarker. However, the connection between biomarker values and tumour incidence rates remains to be clarified. A second effect which occupies the attention of toxicologists and which might have something in common with the first relates to subtle influences on the early development of organisms. Its significance for human health in the longer term is unclear.
- The mode of action of dioxins and dioxin-like compounds has been extensively studied over the past decades. Investigations have provided a fairly clear picture of the relevant signalling pathways (Sch96). Ah receptor-mediated responses are

usually classified as either adaptive, involving the upregulation of genes encoding xenobiotic-metabolizing enzymes, or toxic, involving effects which are inconsistent with an adaptive response and appear to have a negative impact. Only potent Ah receptor agonists seem to be able to elicit these toxic responses.

2.4 Specified questions for discussion following the introductory contributions concerning 'ionising radiation', 'UV radiation', and 'dioxins'

1 Which intracellular and intercellular processes qualify for consideration in assessing the health effects of low levels of exposure?
2 How should 'exposure' be defined with respect to these processes, and which exposures or exposure rates are to be taken as 'low'?
3 When does the evidence base suffice to incorporate such processes into scientific or risk assessment models, and how should this be done?
4 How are models taking modes of action into account to be fitted to epidemiological or experimental data, e.g. what are the perspectives for biomarker epidemiology?
5 Can a priority list be given of issues in need of clarification?

3 The importance of conceptual frameworks

3.1 Biological complexity and ideas for dealing with it

The more detailed and refined our insights into the toxicokinetic and toxicodynamic properties of a particular environmental stressor become, the better will be our predictions of what happens at low levels of exposure, or rather the less uncertain we will feel about them. But this truism involves two major drawbacks. Firstly, there may be a very large number of facts about the stressor in question which in principle qualify for consideration in predicting its low level effect. In the final analysis that may lead to very complicated and time consuming descriptions and derivations. Secondly, there exists a large variety of stressors, which may considerably differ in their biochemical characteristics and mutual interactions. This might entail, among other things, that predictions of the effect of low level exposure to an individual stressor given the simultaneous influence of other stressors pose even greater difficulties.

So, in order to keep things manageable simplifications are unavoidable. Radiobiologists and toxicologists are engaged in selecting mechanisms and processes to be included in the descriptions of the (potential) effects of low level exposures. Often the available data about an individual environmental stressor are the be-all and end-all of the analysis. Approaches attempting to integrate the mode of action of a

particular stressor and the gamut of simultaneously operating endogenous and exogenous (’background’) factors seem to receive relatively little attention. Other branches of biology might be helpful here. For instance, they might provide evidence for the existence of a set of global constraints on possible interactions of diverse mechanisms, irrespective of some of the individual characteristics of a particular stressor. Such constraints could be important for other reasons as well. Data for many stressors are scarce or fragmentary rather than numerous or detailed. Global principles of description might then be a scientifcally justified way to compensate for this lack of data. They might also be used to suggest and defend research priorities.

In theoretical biology interactions between deleterious and defensive mechanisms are studied from a very general perspective (Dou96). An example is what kind of systems are able to produce phenomena which are characterized by non-monotonic exposure-effect curves. Evolutionary biology has as one of its basic ideas that humans like any other organism have a history of adaptation and natural selection. From this point of view it may be argued that there exists a system of checks and balances (homeostasis), but also that with toxic stress physiological costs are enhanced (For96). The possible links between such costs, homeostasis, and adverse effects on health are intriguing. Biogerontology is a field of study where insights are being developed which for a number of reasons may be particularly useful in this respect (Ess95, Kow96). Because attention is focused on, usually multifactorial, physiological effects rather than on the influence of separate stressors, integrative approaches are a natural characteristic of this discipline. Moreover, ageing and late health effects, such as cancer, are often assumed to be tightly coupled processes: as maintenance and repair become increasingly ineffective with age, the incidence of chronic disorders strongly increases.

Two interrelated phenomena just mentioned, viz. the simultaneous influence of various endogenous and exogenous stressors and the multifactorial character of many health effects, cause some experts to argue that linearity of exposure-effect curves might be the rule rather than the exception for environmental stressors (Hei97). This position seems to depend on a number of critical assumptions, in particular that the ‘background’ level of the effects under analysis is non-zero and that defensive countermeasures have already been overwhelmed. The validity of these assumptions is a matter of debate. Differences in susceptibility to environmental stressors between various groups within a population might turn out to be a factor of major importance in this context.

Insights into signalling pathways between cells are rapidly growing. The existence of such types of cell-cell communication is often assumed to be a clear indication for the

defensive capabilities of the human body. But pathways like these might also be involved in pathogenetic processes. Two mechanisms might be a case in point. Firstly, gap junctions, which are formed by proteins called connexins, play a vital role in embryogenesis, cell differentiation, and the coordination of tissue responses. Emerging data gradually show that abnormalities in connexins can lead to various diseases (Pen96). Secondly, the nuclear factor NF-kB turns on genes involved in the body's response to inflammation, infection, and stress (Bae96). Recent experiments have suggested that NF-kB might both block and mediate apoptotic cell death (Lip97). This illustrates the potential complexity of signalling cascades associated with inter- and intracellular processes and draws attention to the opposing forces which might be at work there.

3.2 Questions for discussion following the introductory contributions in sessions 1 and 5

1 Is it possible to formulate some general constraints on descriptions of the effects of low level exposures, for example principles depending on the exposure rate, on the replicative capacity of the cell types under attack, and on the number and nature of stages involved in pathogenesis?
2 How should the influence of simultaneously operating endogenous and exogenous ('background') factors be taken into account?

References

Bae96 Baeuerle PA, Baltimore D. NF-kB: ten years after. Cell 1996; 87 : 13-20.

Bir95 Birgelen APJM van, Smit EA, Kampen IM, a.o. Subchronic effects of 2,3,7,8-TCDD or PCBs on thyroid hormone metabolism: use in risk assessment. Eur J Pharmacol 1995; 293 : 77-85.

Bog97 Bogen KT. Do U.S. county data disprove linear no-threshold predictions of lung cancer risk for residential radon? - a preliminary assessment of biological plausibility. Human and Ecological Risk Assessment 1997; 3:157-86.

Dou96 Doucet PG. A feedback model for hormesis (mimeo, Amsterdam, 1996)

Ess95 Esser K, Martin GM (eds). Molecular aspects of ageing. Chichester, 1995.

For96 Forbes VE, Calow P. Costs of living with contaminants: implications for assessing low-level exposures. BELLE Newsletter 1996; 4: 1-8.

Gru96 Gruijl FR de. Photobiology of photocarcinogenesis. Photochem and Photobiol 1996; 63 : 372-5.

Hei97 Heitzmann M, Wilson R. Low-dose linearity: the rule or the exception? BELLE Newsletter 1997; 6(1) : 2-8.

Koh96 Kohn MC, Sewall CH, Lucier GW, Portier CJ. A mechanistic model of effects of dioxin on thyroid hormones in the rat. Toxicol Appl Pharmacol 1996; 136 : 29-48.

Kow96 Kowald A, Kirkwood TBL. A network theory of ageing: the interactions of defective mitochondria, aberrant proteins, free radicals and scavengers in the ageing process. Mutat Res 1996; 316 : 209-36.

Lip97 Lipton SA. Janus faces of NF-kB: neurodestruction versus neuroprotection. Nature Medicine 1997; 3 : 20-1.

Lue96 Luebeck EG, Cutis SB, Cross FT, Moolgavkar SH. Two-stage model of radon-induced malignant lung tumors in rats: effects of cell killing. Radiat Res 1996; 145: 163-73.

Moo90 Moolgavkar SH, Cross FT, Luebeck G, a.o. A two-mutation model for radon-induced lung tumors in rats. Radiat Res 1990; 121: 28-37.

Mut96 Mutation Research. The adaptive response to very low doses of ionizing radiation. Special issue. Mutat Res 1996; 358 : 125-246.

NRP95 National Radiological Protection Board. Risk of radiation-induced cancer at low doses and low dose rates for radiation protection purposes. Chilton, Didcot, Oxon 1995.

Pen96 Pennisi E. Genes, junctions, and disease at cell biology meeting. Science 1996; 274 : 2008-9.

Sch96 Schmidt JV, Bradfield CA. Ah receptor signaling pathways. Annu Rev Cell Dev Biol 1996; 12 : 55-89.

Sel97 Selgrade MK, Repacholi MH, Koren HS. Ultraviolet radiation-induced immune modulation: potential consequences for infections, allergic, and auto-immune disease. Environ Health Perspect 1997; 105 : 332-4.

UNS94 UNSCEAR. Annex B. Adaptive responses to radiation in cells and organisms, in: Sources and effects of ionizing radiation. 1994 Report to the General Assembly, with annexes. New York, 1994.

Yar96 Yarosh DB, Kripke ML. DNA repair and cytokines in antimutagenesis and anticarcinogenesis. Mutat Res 1996; 350 : 255-60.

Annex **F**

Conference programme

October 19, Sunday

- 16.00 - 17.00 *Reception*

Session I. Heuristic overture
(For background information see section 3.1 of the note (Annex E))

- 17.00 - 18.30 *Opening address*
  JA Knottnerus (chairman)

  Aim and structure of the working conference
  EJ Schoten

  Low level effects from a theoretical perspective
  P Doucet

  Low level effects from the perspective of evolutionary biology
  SALM Kooijman

  Low level effects from the perspective of biogerontology
  J Vijg

Possibilities and impossibilities of environmental epidemiology
B Brunekreef

- 18.30- 19.30 *Discussion*

  (For questions see section 3.2 of the note)

- 19.30 - 21.00 *Dinner*

October 20, Monday

- 09.00 - 09.05 *Technical information*

Session II. Ionising radiation
(For background information see section 2.1 of the note)

- 09.05 - 10.15 *Introduction*
  BA Bridges (chairman)

  Significance of data on repair mechanisms
  R Cox

  Potential contributions from biomarker epidemiology
  R Cox

  Insights into adverse effects
  BA Bridges

- 10.15 - 11.30 *Discussion*

  (For questions see section 2.4 of the note)

- 11.30 - 12.30 *Break, Lunch*

Session III. UV radiation
(For background information see section 2.2 of the note)

- 12.30 - 13.45 *Introduction*
  JC van der Leun (chairman)

Cellular effects and repair mechanisms
LHF Mullenders

Intercellular effects and repair mechanisms
SE Ullrich

Combining epidemiological and mechanistic information
FR de Gruijl

- 13.45 - 15.00 *Discussion*

  (For questions see section 2.4 of the note)

- 15.00 - 16.00 *Break*

Session IV. Dioxins
(For background information see section 2.3 of the note)

- 16.00 - 17.15 *Introduction*
  HG Neumann (chairman)

  Using in vitro and in vivo data on carcinogenicity
  M van den Berg

  Using in vitro and in vivo data on developmental effects
  EK Silbergeld

  Combining epidemiological and mechanistic information
  EK Silbergeld

- 17.15 - 18.30 *Discussion*

  (For questions see section 2.4 of the note)

- 18.30 - 20.00 *Dinner*

October 21, Tuesday

- 09.00 - 09.05 *Technical information*

Session V. Scientific possibilities and limitations

- 09.05 - 10.00 *Introduction*
  JE Trosko (chairman)

  Synthesis with a view to modelling
  CJ Portier

  Significance of cellular and intercellular processes
  JE Trosko

- 10.00 - 12.00 *Discussion on the basis of a handout made at the working conference*

  (For questions see also section 3.2 of the note)

- 12.00 - 13.30 *Break, lunch*

Session VI. Perspectives for risk assessment

- 13.30 - 15.30 *Introduction*
  JA Knottnerus (chairman)

  Concluding discussion about questions and statements in the handout

  Closing address

Annex **G**

Participants and Scientific Advisory Committee

The following experts participated in the working conference:

- dr M van den Berg; Research Institute of Toxicology; University of Utrecht; The Netherlands
- dr BA Bridges; Medical Research Council Cell Mutation Unit; University of Sussex; Brighton, United Kingdom
- dr B Brunekreef; Department of Health Studies; Agricultural University of Wageningen; The Netherlands
- dr KH Chadwick; Radiation Protection Research Unit; Directorate General XII of the European Commission; Brussels, Belgium
- dr R Cox; National Radiological Protection Board; Chilton, Didcot, United Kingdom
- dr P Doucet; Department of Theoretical Biology; Free University of Amsterdam; The Netherlands
- dr WH Farland; Environmental Protection Agency; Washington, United States
- dr VJ Feron; Toxicology Division; TNO Nutrition and Food Research Institute; Zeist, The Netherlands
- dr FR de Gruijl; Department of Dermatology; University Hospital of Utrecht; The Netherlands
- dr JHJ Hoeijmakers; Department of Cell Biology and Genetics; Erasmus University Rotterdam; The Netherlands
- dr SALM Kooijman; Department of Theoretical Biology; Free University of Amsterdam; The Netherlands

- dr E Lebret; Department for Chronic Diseases and Environmental Epidemiology; National Institute of Public Health and the Environment; Bilthoven, The Netherlands
- dr JC van der Leun; Department of Dermatology; University Hospital of Utrecht; The Netherlands
- dr PHM Lohman; Department of Radiation Genetics and Chemical Mutagenesis; Leiden University Medical Center; The Netherlands
- dr LHF Mullenders; Department of Radiation Genetics and Chemical Mutagenesis; Leiden University Medical Center; The Netherlands
- dr HG Neumann; Department of Toxicology; University of Würzburg; Germany
- mrs MNEJG Philippens; Ministry of Housing, Spatial Planning, and the Environment; The Hague, The Netherlands
- dr CJ Portier; National Institute of Environmental Health Sciences; Research Triangle Park, United States
- dr W Seinen; Research Institute of Toxicology; University of Utrecht; The Netherlands
- dr EK Silbergeld; Department of Epidemiology and Preventive Medicine; University of Maryland at Baltimore; United States
- dr W Slob; Laboratory for Health Effects Research; National Institute of Public Health and the Environment; Bilthoven, The Netherlands
- dr JE Trosko; Department of Pedriatics and Human Development; Michigan State University; East Lansing, United States
- dr SE Ullrich; Department of Immunology; MD Anderson Cancer Center; Houston, United States
- dr PF de Vries Robbé; Department of Medical Informatics and Epidemiology; University of Nijmegen; The Netherlands
- dr J Vijg; Harvard Institute of Medicine; Harvard University; United States
- dr JD Wilson; Resources for the Future; Washington, United States

Participants from the Health Council were:
- dr ASAM van der Burght
- dr JA Knottnerus, Vice President
- dr WF Passchier
- dr E van Rongen
- mr EJ Schoten, Scientific Secretary
- dr PW van Vliet

Drs Van der Burght, Van Rongen, and Van Vliet helped draw up the minutes of the conference. Mrs MFC van Kan gave secretarial assistance.

Members of the Scientific Advisory Committee for the conference were:

- dr BA Bridges; Medical Research Council Cell Mutation Unit; University of Sussex; Brighton, United Kingdom
- dr EJ Calabrese; Northeast Regional Environmental Public Health Center; University of Massachusetts; Amherst, United States
- dr JHJ Hoeijmakers; Department of Cell Biology and Genetics; Erasmus University Rotterdam; The Netherlands
- dr TBL Kirkwood; School of Biological Sciences and Department of Geriatric Medicine; University of Manchester; United Kingdom
- dr SALM Kooijman; Department of Theoretical Biology; Free University of Amsterdam; The Netherlands
- dr PHM Lohman; Department of Radiation Genetics and Chemical Mutagenesis; Leiden University Medical Center; The Netherlands
- dr HG Neumann; Department of Toxicology; University of Würzburg; Germany
- dr CJ Portier; National Institute of Environmental Health Sciences; Research Triangle Park, United States
- dr JE Trosko; Department of Pediatrics and Human Development; Michigan State University; East Lansing, United States

HELLE

Gezondheidseffecten van lage blootstellingniveaus

Hoofdstuk **1**

De aard van het probleem

De Gezondheidsraad is nauw betrokken bij de wetenschappelijke onderbouwing van blootstellingsnormen voor stoffen en straling ter bescherming van de volksgezondheid. In de loop der jaren heeft de Raad bijgedragen aan de formulering van principes en procedures, zowel voor carcinogene als voor niet-carcinogene agentia . Bij de afleiding van gezondheidskundige advieswaarden draait de discussie als regel om de vraag welke extrapolatiemethodes in aanmerking komen (wat valt te concluderen uit gegevens over hoge blootstelling en over proefdieren?). In het algemeen schort het namelijk aan rechtstreekse gegevens over gezondheidseffecten bij lage niveaus van blootstelling. Effecten bij die niveaus laten zich zelden detecteren via het gangbare dierexperimentele of epidemiologische onderzoek: daarvoor schiet het vermogen van deze analyse-instrumenten om ‘signaal’ van ‘ruis’ te onderscheiden meestal tekort. Annex B bij dit advies bevat een korte schets van de moeilijkheden en van de ingeburgerde manieren om daaraan het hoofd te bieden.

Toch bestaat de hoop dat de veronderstelde zwakke signalen, indien aanwezig, langs andere weg kunnen worden opgevangen. Men zou dan dieper moeten graven, dat wil zeggen moeten trachten na te gaan wat zich op onderliggende niveaus van biologische organisatie afspeelt wanneer organismen worden blootgesteld aan lage doses straling of stoffen. De moleculaire en de celbiologie reiken diverse methodes en technieken aan waarmee processen in cellen in kaart gebracht kunnen worden. Als gevolg daarvan groeit het inzicht in de moleculaire en cellulaire effecten van blootstelling aan agentia, dat wil zeggen in de werkingsmechanismen die aan de gezondheidseffecten ten grondslag liggen. De Gezondheidsraad achtte vorig jaar de tijd rijp voor een inventarisatie

van de stand van wetenschap op dit terrein. Daartoe werd van 19 tot en met 21 oktober 1997 een internationale werkconferentie georganiseerd met als titel: ‘Health Effects of Low Level Exposures: Scientific Developments and Perspectives for Risk Assessment’.

Kernvraag was in hoeverre de soms snel groeiende kennis over moleculaire en cellulaire effecten het verhoopte houvast biedt voor extrapolatie. Verschillende deelvragen waarover al langere of kortere tijd een debat woedt, kwamen tegen dat decor aan de orde. Een van de voornaamste kwesties betrof de gangbare, maar steeds vaker ter discussie gestelde tweedeling tussen stochastisch en niet-stochastisch werkende agentia, en het daarmee corresponderende onderscheid tussen blootstelling-effectrelaties zonder en met een drempel (zie Annex B voor een beknopte toelichting). Ook werd van gedachten gewisseld over wat dikwijls wordt aangeduid als hormese: lage blootstellingsniveaus zouden de gezondheid kunnen bevorderen. Om de vele facetten van de thematiek belicht te krijgen, waren deskundigen uit diverse vakgebieden uitgenodigd. Verder waren drie agentia als kristallisatiepunten gekozen voor het algemenere debat: ioniserende straling, ultraviolette (UV) straling en dioxinen.

In het voorliggende signalement wordt aandacht gevraagd voor enkele zaken die tijdens de discussies over de zoëven aangeduide kernvraag naar voren kwamen. Diverse detailkwesties en de bredere context van de beschouwingen worden uitvoeriger beschreven in het bijgevoegde verslag van de conferentie (Annex C) en in aan het verslag gehechte achtergronddocumenten (Annexes D en E). Wat volgt is een reeks overwegingen met betrekking tot de wetenschappelijke basis voor de afleiding van advieswaarden, bezien in het licht van de vigerende procedures en tegen de achtergrond van het werk van de Gezondheidsraad. Bij de voorbereiding van de hierna volgende opmerkingen en aanbevelingen zijn verscheidene Nederlandse deskundigen geraadpleegd (zie Bijlage A).

Hoofdstuk **2**

De stand van wetenschap

Naar de mening van de deelnemers laat de kernvraag van de conferentie zich niet in algemene zin beantwoorden. Voor sommige agentia, zoals ioniserende straling, UV straling en dioxinen, is verhoudingsgewijs veel bekend over werkingsmechanismen. Maar zelfs dan staan verscheidene problemen een verreikende kwantitatieve modellering van blootstelling-effectrelaties op moleculair en celbiologische grondslag in de weg. Zo is het inzicht in de ontstaanswijze van ziekten en aandoeningen die mede veroorzaakt zouden kunnen worden door de bedoelde agentia, over het algemeen nog erg beperkt. Ook als de moleculair-biologische fundamenten grotendeels zijn blootgelegd, kent men nog niet alle stappen in het traject van gebeurtenissen binnen cellen naar manifeste gezondheidsschade. Tijdens de werkconferentie werd er meermalen op gewezen dat voor een goed begrip verschillende niveaus van biologische organisatie in beschouwing genomen moeten worden: onmisbaar als de bestudering van moleculair-biologische processen mag zijn, meer fysiologisch georiënteerd onderzoek speelt eveneens een belangrijke rol. Anderzijds laten de zoëven bedoelde problemen met betrekking tot de ontstaanswijze van ziekten zich in zoverre relativeren, dat niet per se alle processtappen steeds haarfijn bekend hoeven te zijn. Gesteld men weet dat bepaalde moleculaire of fysiologische biomarkers — waarover straks meer — eenduidig samenhangen met bepaalde vormen van blootstelling en van gezondheidseffecten. Dan kan een nauwkeurige kartering van het tussengelegen traject voor de risicobeoordeling achterwege blijven. Overigens kan dergelijke informatie voor andere doeleinden, bijvoorbeeld voor de ontwikkeling van medische interventies, wel heel waardevol zijn.

Als men vanuit de invalshoek van risicofactoren naar pathogenetische processen kijkt, doemen verdere moeilijkheden op. De conferentiedeelnemers gaven aan dat zich onder invloed van xenobiotische agentia een scala aan fenomenen in cellen kan voordoen. Daartoe behoren veranderingen in genexpressie, mutaties en celdood via apoptose (geprogrammeerde afsterving) of necrose (andersoortige afsterving). Ook is het mogelijk dat zulke interacties geen duidelijke sporen nalaten. Welke veranderingen specifieke agentia zoal teweeg kunnen brengen, is vaak onvoldoende bekend. Vervolgens tast men niet zelden in het duister over de mogelijke invloed van die veranderingen op de gezondheid, of het daarbij nu gaat om een verhoogde kans op kanker, om versnelde veroudering of om ontregeling van bepaalde orgaanfuncties. Bij dit alles komt nòg een obstakel: hoe cellulaire processen — en hun resultante — precies afhangen van de mate en het tempo van blootstelling, is grotendeels in nevelen gehuld.

Er zijn dus vragen te over en meestal betrekkelijk weinig gegevens voor de beantwoording daarvan, zelfs als algemene informatie over moleculaire en cellulaire processen in ruime mate ter tafel ligt. Mechanistische modellering van blootstelling-effectrelaties lijkt de eerstkomende tijd nog buiten bereik te blijven, althans modellering ‘over de hele linie’, dat wil zeggen van complete pathogenetische processen. Maar kennis over bepaalde deelprocessen bij blootstelling aan bepaalde xenobiotische agentia komt wel degelijk in een aanhoudende stroom beschikbaar. Zoals gezegd kan dat voor risicobeoordeling soms toereikend zijn.

Interessante ontwikkelingen doen zich onder meer voor op het terrein van de toxicokinetiek en -dynamiek. Door na te gaan hoe stoffen zich bij opname in het lichaam gedragen, is het mogelijk meer zicht te krijgen op de biologisch relevante (effectieve) blootstelling. Met behulp van zogeheten PBPK/PD-modellen (PBPK/PD: *physiologically based pharmacokinetic and pharmacodynamic*) proberen toxicologen de betrokken verdelings- en omzettingsprocessen expliciet en systematisch te beschrijven. Bij dioxinen bijvoorbeeld wordt daar de laatste jaren nogal wat onderzoek naar gedaan. Men ziet validering van dit soort modellen alom als een onderzoeksprioriteit en verwacht dat bijvoorbeeld de problemen van extrapolatie ‘van dier naar mens’ zo verminderd kunnen worden.

In het verlengde van de zojuist geschetste ontwikkelingen ligt veelbelovend onderzoek naar biomarkers voor inwendige blootstelling en voor verhoogde gevoeligheid. Zo zouden mensen met bepaalde genetische eigenschappen eerder of sterker dan anderen de nadelige gevolgen van blootstelling aan bepaalde agentia kunnen ondervinden. Komt men dergelijke biomarkers op het spoor en slaagt men erin daarvan in fenomenologisch onderzoek gebruik te maken, dan kan de zeggingskracht van de analyses toenemen. Biomarkers voor vroege, dat wil zeggen aan manifeste gezondheidsschade voorafgaande, effecten laten waarschijnlijk langer op zich wachten.

Ook trends op het terrein van de transgenese (het overbrengen van gewenste erfelijke eigenschappen naar het genoom van een proefdier) bieden perspectief. Door gerichte gen-inactivatie in muizen is het mogelijk om specifiek één of meer cellulaire processen, bijvoorbeeld DNA-herstel en metabolisme van chemische stoffen, uit te schakelen. Zodoende kan men analyseren hoe dergelijke processen het effect van blootstelling aan xenobiotische agentia beïnvloeden. Vanwege de sterk verhoogde gevoeligheid van sommige muismutanten voor bepaalde stoffen is ook het effect van lage doseringen soms direct meetbaar. Verder zullen transgene muizen die voorzien zijn van gevoelige systemen voor de detectie van mutaties, in de nabije toekomst het inzicht in het effect van blootstelling aan genotoxische agentia kunnen vergroten.

Dan is er nog de voortgaande opmars van de informatietechnologie, die de analyse en synthese van allerlei voorliggende gegevens kan vereenvoudigen en verfijnen. Tijdens de conferentie viel te beluisteren dat de op dat gebied bestaande mogelijkheden misschien nog te weinig benut worden. De statistische bewerking van uitkomsten van dierexperimenteel en epidemiologisch onderzoek, waar mogelijk aangevuld met informatie over werkingsmechanismen, kan nadere aanwijzingen verschaffen over de mate van onzekerheid waarmee de bepaling van blootstelling-effectrelaties en de afleiding van advieswaarden verbonden zijn. Zulke analysemethodes kunnen soms uitsluitsel geven over de waarschijnlijkheid van het bestaan van een drempeldosis voor het optreden van bepaalde effecten.

Een vraagstuk dat aparte vermelding verdient, betreft de feitelijke omstandigheden van blootstelling. Steeds is in de praktijk sprake van een gecombineerde invloed van een breder of smaller spectrum van endogene en exogene factoren, met deels overeenkomstige werkingsmechanismen. Een voorbeeld is de productie van zogeheten vrije radicalen (bepaalde reactieve moleculen) door het normale zuurstofmetabolisme en door blootstelling aan ioniserende straling. Op de conferentie kwamen twee kwesties aan de orde die met dat gegeven verband houden.

Ten eerste kan men zich afvragen of het correct en zinnig is blootstelling-effectrelaties af te leiden voor afzonderlijke agentia. Die vraag roept echter onmiddellijk een wedervraag op, namelijk welke uitgangspunten dan zijn te hanteren bij de bepaling van relaties tussen combinatie-blootstelling en gezondheidseffecten. Een principiële oplossingsrichting tekende zich tijdens de conferentie nog niet af.

Ten tweede is er de mogelijkheid van hormese. Sommige onderzoekers sluiten niet uit, of vinden het zelfs plausibel, dat blootstelling aan een specifiek agens onder omstandigheden bepaalde reactiemechanismen mobiliseert die de netto schade van de combinatie-blootstelling verminderen. Naar het oordeel van de conferentiedeelnemers ontbreken daarvoor echter totnogtoe overtuigende aanwijzingen.

Hoofdstuk **3**

De implicaties voor risicoanalyse

Het voorgaande leidt tot de conclusie dat de huidige beoordelingssystematiek op bepaalde onderdelen en onder bepaalde omstandigheden verfijnd kan worden. Ontwikkelingen met betrekking tot bijvoorbeeld PBPK/PD-modellering, biomarkers voor variaties in gevoeligheid en modelleringsmethodieken, bieden kansen voor een nadere onderbouwing van elementen of modules die een plaats hebben binnen de vigerende systematiek. Men kan denken aan beter gefundeerde veiligheids- of extrapolatiefactoren waarmee mogelijke verschillen in gevoeligheid tussen en binnen species verdisconteerd worden. Idealiter laten zulke factoren zich geheel vervangen door (deel)modellen die de bedoelde variaties expliciet beschrijven. Meer in het algemeen valt te verwachten dat de relaties tussen componenten van het zogeheten integraal toxiciteitsprofiel, zoals beschreven en toegelicht in het Gezondheidsraadadvies ‘Toxicologische advieswaarden voor blootstelling aan stoffen’ (1996/12), met behulp van de hier bedoelde analysetechnieken beter in kaart gebracht kunnen worden.

Wat betreft de omstandigheden waaronder diepere analyses gerechtvaardigd lijken, verdient de doelmatigheid van de risicobeoordeling aandacht. Doorwrochte analyses in de zojuist bedoelde zin zijn arbeidsintensief en kostbaar. Het valt te overwegen ze het eerst te beproeven bij maatschappelijk prioritaire agentia. Criteria als de plausibiliteit van schadelijkheid bij feitelijk te verwachten blootstellingsniveaus, de omvang van de blootgestelde populatie, de ernst van de effecten, de mogelijkheid van risicovermindering en de grootte van de meespelende economische belangen zouden bij de selectie van die agentia behulpzaam kunnen zijn. Zo’n selectie is ook om een andere reden van belang: de risicobeoordeling van bestaande stoffen op de Europese markt verloopt nog-

al traag. Er zullen ook tijd en middelen moeten worden gereserveerd om daarin verbetering te brengen.

Als het bij een risicobeoordelingsvraagstuk om diepgang en maatwerk gaat, kan een goed geregisseerde inbreng van verschillende deskundigen niet worden gemist. Zij zullen per geval, in onderling overleg, moeten bezien welk model het geheel aan voorliggende gegevens het best beschrijft. Op dit moment is het moeilijk een uitspraak te doen over de generaliseerbaarheid van zulke modellen. De conferentiesessies gewijd aan ioniserende straling, UV straling en dioxinen illustreerden dit probleem (zie Annex C). In ieder geval zijn nu nog geen algemene aanbevelingen mogelijk met betrekking tot uitgangspunten voor mechanistische modellering of met betrekking tot de invloed van homeostatische controleprocessen. Kortom, de ervaring zal moeten leren in welke zin en hoe snel de huidige beoordelingssystematiek zich laat verfijnen.

In het Werkprogramma 1999 van de Gezondheidsraad zijn, onder de kop ‘Uitgangspunten voor gezondheidskundige advieswaarden’, vijf thema’s opgenomen die nauw verband houden met het voorgaande: (1) het opstellen van een integraal toxiciteitsprofiel; (2) het gebruik van epidemiologische gegevens bij het opstellen van zo’n profiel; (3) het toepassen van de zogeheten ‘benchmark dose’-benadering (de benchmark dose is de onderste statistische betrouwbaarheidsgrens van de blootstelling die behoort bij een bepaald responsniveau); (4) het gebruik van veiligheidsmarges; en (5) het omgaan met combinatie-blootstelling. In Nederland vindt ook onderzoek naar een aantal van deze onderwerpen plaats, bij universitaire vakgroepen, in onafhankelijke onderzoekslaboratoria, bij de overheid en vanuit de industrie. De soms al intensieve samenwerking tussen deze en buitenlandse instanties zal de kwaliteit en doelmatigheid van de risicobeoordeling in ons land zeker ten goede komen.

A Totstandkoming van het advies

Bijlage

Bijlage **A**

Totstandkoming van het advies

Het advies is voorbereid door Eert Schoten, secretaris bij de Gezondheidsraad, na raadpleging van de volgende deskundigen:

- dr ir B Brunekreef; hoogleraar gezondheidsleer; Landbouwuniversiteit Wageningen
- dr VJ Feron; hoogleraar biologische toxicologie; Universiteit Utrecht
- dr JHJ Hoeijmakers; hoogleraar moleculaire biologie; Erasmus Universiteit, Rotterdam
- dr ir PHM Lohman; hoogleraar stralengenetica en chemische mutagenese; Rijksuniversiteit Leiden