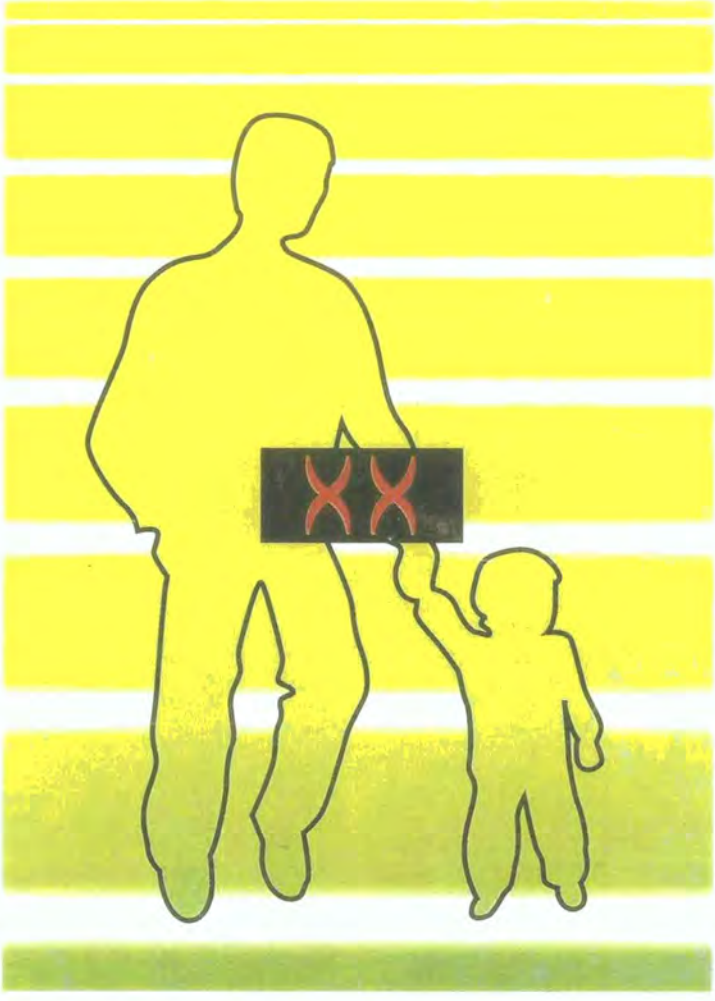
ASSESSING THE RISK OF GENETIC DAMAGE



UNEP

ICPEMC

UNITED NATIONS ENVIRONMENT PROGRAMME

INTERNATIONAL COMMISSION FOR PROTECTION AGAINST ENVIRONMENTAL MUTAGENS AND CARCINOGENS

Assessing the Risk of Genetic Damage

Editors: D.J. Brusick, H.N.B. Gopalan, E. Heseltine, J.W. Huismans and P.H.M. Lohman

UNEP United Nations Environment Programme

ICPEMC International Commission for Protection against Environmental Mutagens and Carcinogens

Hodder & Stoughton
GABORONE LONDON SYDNEY AUCKLAND

First published in 1992 by
Hodder & Stoughton
Botswana
PO Box 40335
Gaborone

United Nations Environment Programme
Assessing the Risk of Genetic Damage
Nairobi, 1992

Part of a project sponsored by Directorate-General XII,
Commission of the European Communities

Text and artwork : Communication in Science,
Chantal Levraut et Christophe Delestras - France

ISBN 92-807-1339-6

Contents

Foreword

Chemicals have contributed in no small measure to increasing the standard of living of populations around the world. They play a major role in the development of human societies: in agricultural production, in health care, in industry, in transport, in housing and in consumer goods. In recent years, concern has increased over the widespread distribution of chemicals stemming from human activities and their potentially harmful effects on humans and on the ecosystems that sustain us.

Chemicals, as well as ionizing and ultraviolet radiation, can induce permanent genetic alterations in all organisms. These changes, termed mutations, usually have deleterious effects on individuals themselves or on their descendants. The implications of genetic alterations are wide-ranging. In human populations, they may increase the incidences of cancer and genetic diseases. In non-human biota, they may alter the essential balance of an ecosystem or change the virulence of a human pathogen. The release into the environment of a genetically engineered species may have similar results.

Current knowledge about the effects of such agents on human health is limited, and data on their effects on other living organisms are practically non-existent. In order to assess the role of genetic toxicology in determining risks to the genetic integrity of present and future generations of human and non-human biota, the United Nations Environment Programme (UNEP) initiated a joint study with the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC).

The major objective of the study was to develop a series of principles and recommendations that may serve as the basis for assessing the genotoxic hazards of environmental agents. It was considered that such principles and recommendations would be useful in setting priorities for the regulation and control of the

vast array of chemicals to which humans are exposed in the environment; in formulating guidelines or regulations to mitigate the genetic effects of environmental agents; and in designing appropriate mechanisms to minimize the effects of such agents on both humans and other living organisms.

This book is based on the working papers that resulted from the study. It is hoped that it will contribute to creating awareness among decision makers and the public of the risks posed by genotoxic agents to human health and that of the environment, so that not only will appropriate preventive measures be taken but also knowledge in this area will continue to improve, with the ultimate aim that the production and use of such agents will be reduced to a minimum or stopped.

Mostafa Kamal Tolba
Executive Director, UNEP

Preface

The management of toxicological risks implies the capacity to control exposure to toxic agents within acceptable safety limits. Effective control depends on prior detection and identification of hazards and the ability to estimate or monitor exposure. Quantitative relationships between low-level exposures to one or several agents and the resulting health effects must also be determined.

Understanding of the underlying mechanisms by which chemical, physical and biological agents produce adverse effects on health and the environment, gained during the last two decades through studies of genetic toxicology, should be involved in the risk assessment process. In addition, efforts should be directed to increasing this understanding and to developing the best methods for applying the knowledge to risk assessment. Such efforts will lead to more realistic determinations of risk, and, if these risks are communicated properly to decision makers and the public, to more scientific risk management, with the most appropriate results.

These concerns led to the initiation of a joint study entitled, 'Assessment of Comparative Risk Associated with Exposure of Humans and Non-human Biota to Genotoxic Agents', between the United Nations Environment Programme (UNEP) and the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC).

ICPEMC was founded in 1977 and is associated with the International Association of Environmental Mutagen Societies. The Commission consists of eminent scientists in the field of mutagenicity. Its objectives are to identify and promote scientific principles and to make recommendations that may serve as the basis for guidelines and regulations designed to minimize the deleterious effects of the interaction of chemicals with genetic material. The Commission's two main activities are (i) the preparation of authoritative, critical reviews of the current body

of knowledge which may serve in establishing priorities for further research or regulatory action; and (ii) the identification of substances and situations that may entail a significant risk to humans. The study that resulted in this book is an example of ICPEMC's activities.

The initial outlines and objectives for the joint study were prepared at a UNEP/ICPEMC meeting in St Petersburg, Russia, in 1989. The Steering Group for the study consisted of M.L. Mendelsohn (Chairperson), J. Ashby and P.H.M. Lohman. Five working groups, consisting of ICPEMC Commissioners and other experts, were constituted to draft background documents on: hazard identification (Chairperson, D.M. DeMarini); exposure assessment (D.W. Layton); dose and effect assessment (J. Favor); risk characterization (J. Lewtas); and environmental monitoring (J.T. MacGregor). The working groups met twice: at Pizay, France, and at Harper's Ferry, USA. The working papers and an overview were finalized at Pizay in January 1992 and will be the subject of a separate publication.

This book is based on those documents. It is part of an international effort to reduce the risk posed by chemical and physical agents to long-term human and environmental health.

H.N.B. Gopalan
Programme Officer
Environmental Management

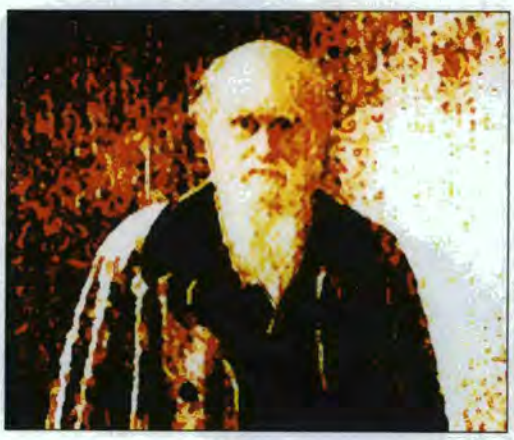
J.W. Huismans
Director
International Register of
Potentially Toxic Chemicals

Genetic damage, in the form of *mutations*—changes in the structure of DNA—can have serious effects on human health, including a wide range of hereditary diseases, cancer, congenital anomalies and even reduced life expectancy. The induction of damage to the germ line and to the mechanisms that control cellular division in living organisms therefore has substantial consequences for health and for the environment.

Because human beings are part of a mutually interdependent ecosystem, genetic damage that alters or eliminates species that are critical to complex food chains may result in imbalances that affect the well-being and possibly the survival of certain populations. The maintenance of domesticated species of plants and animals depends on our ability not only to develop but also to protect the integrity of the genetic make-up of those species from *mutagens* present in the environment

Genetic damage (mutations) can be induced in the hereditary molecules of plants and animals by both natural and man-made mutagens. Some chemicals present in the environment are beneficial or even essential for life, whereas others are toxic to living organisms by reacting with the molecules that are responsible for their reproduction and the transmission of characteristics. Natural mutagens, such as ionizing radiation, sunlight (ultra-violet radiation) and products resulting from the burning of forests and fossil fuels, pose risks to the hereditary machinery of organisms. In addition to these natural mutagens, however, are an increasing number of chemicals found in the environment which are either man-made or are derivatives or reaction products of man-made chemicals. The number of man-made chemicals being released into the environment is increasing, owing to heavier use of chemicals in manufacturing, agriculture and transportation; but only about 10% have been evaluated adequately for their ability to cause mutations.

Mutations are a normal part of the evolution of plant and animal species. They result in the genetic heterogeneity that is essential for the survival of species in the face of environmental change. Charles Darwin's theory of evolution is based partly on the existence of environmentally induced genetic variability.



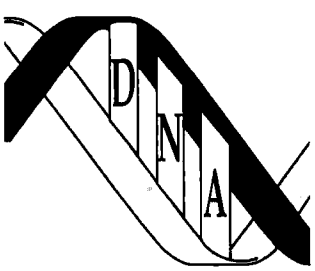
Charles Darwin

Background, or spontaneous, mutations occur in all organisms, probably as a result of damage caused by naturally occurring mutagens or errors that occur during replication of hereditary molecules during cell division.

Spontaneous mutations not only provide the heterogeneity necessary for evolution but also contribute to the incidence of recurring diseases, known as the 'genetic burden' of a species. The 'genetic burden' of the human species is currently estimated to be about 10% (one affected individual per 10 live births). This is a relatively heavy 'burden'; it accounts for at least 25% of the costs of health care in developed countries. In view of the economic impact of health care alone, it would seem prudent to develop methods for identifying mutagens and for evaluating the risk they pose to heredity, in relative or absolute terms.

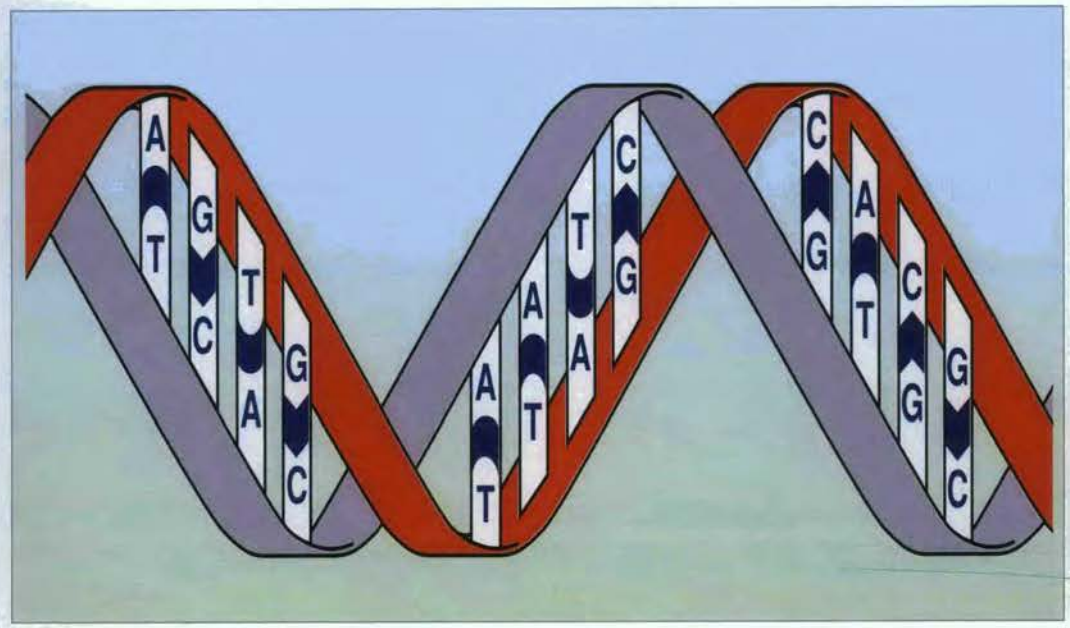
The pool of genes that will form all future generations of plants and animals on the earth currently resides in the germ lines of existing individuals. This pool must be protected from mutagenic damage, to prevent deterioration of health and ensure the survival of the species that make up the global ecosystem. Protection of the gene pool requires identification of the risks associated with actual exposure to mutagens. This process is called *risk assessment*. It comprises integration of agent classification, exposure assessment, extrapolation of information on the potency of the agent to the human situation and risk characterization. Once the relative or absolute risk has been estimated, decisions can be taken about the appropriate measures for managing that risk.

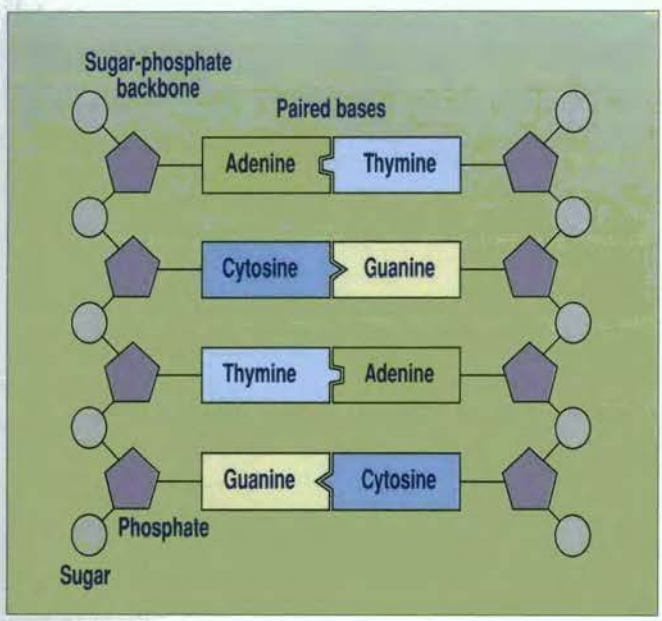
What is Mutation?



DEOXYRIBONUCLEIC ACID, or DNA, is vital to life and is its most basic chemical component. It contains complete, coded instructions for the formation, function and reproduction of virtually all forms of life.

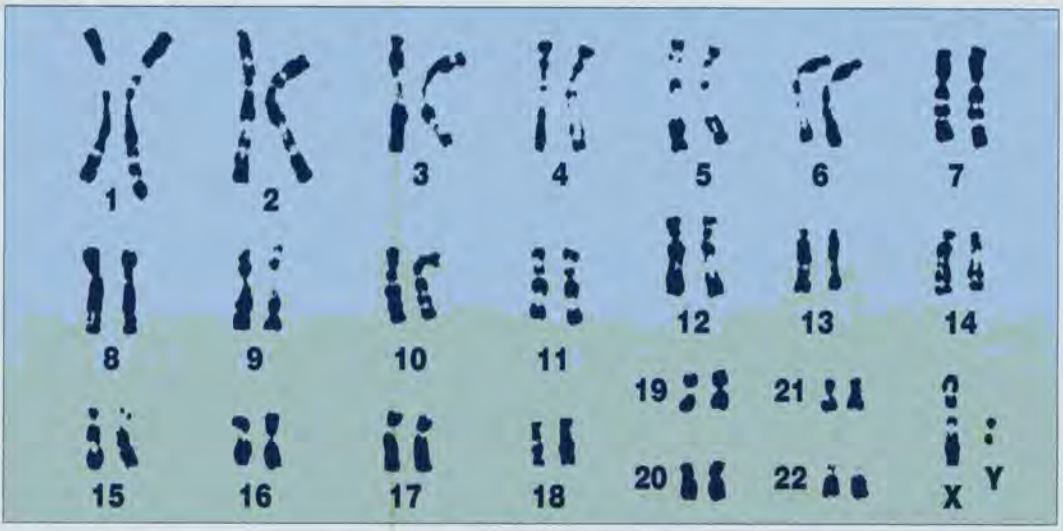
The maintenance of the integrity of DNA in all cellular organisms on the planet is essential.





Sense and antisense: the strands of DNA that form the double helix are bound together by 'base pairing': adenine always binds to thymine, and guanine to cytosine. The strands are said to be complementary to one another, but a cell normally uses only one of the strands, the 'sense' strand, to make proteins.

Environmental mutagens—either chemicals or radiations—are agents that are capable of interacting with DNA, directly or indirectly, to alter the integrity and the information present in the genetic material.



The 46 chromosomes that are found in all human cells

The genetic information system of higher animals is organized into **chromosomes**, which consist of DNA and proteins. The primary information is contained in the sequence of nucleotide bases that make up DNA. A mutagen can cause addition or deletion of a base or substitution of one base for another and thus change the function of a single gene. Such changes are called *gene* or *point mutations*. Mutagens can also break the physical structures of chromosomes to produce fragments, which may rejoin themselves haphazardly, and genetic material may be lost or gained. This type of genetic damage is known as *chromosomal mutation*. A third kind of genetic damage involves a change in the number of chromosomes. Each species is characterized by a defined number of chromosomes, and any departure from that number affects the genetic integrity of the organism. Such departures are referred to as *aneuploidy*.

The detrimental effects of exposure to environmental mutagens may be divided into two broad categories:

—those resulting from genetic damage to the cells of the germ line

—those resulting from genetic damage to somatic cells

The *germ cells* are the reproductive cells that ensure the continuation of a species. All the other cells in the body are referred to as *somatic cells*. A mutation in a somatic cell will therefore affect only the individual in which it occurs. Mutations in germ cells, however, may affect not only individuals but also their descendants. For the purposes of this document, the term 'genetic risk' includes risk to both somatic and germ cells.

Inherited disease

The health of subsequent generations is dependent, first and foremost, on inheriting a *genome* (all the genetic material of an organism) that does not contain mutant genes that might result in malformation, disease or untimely death.

Many human inherited diseases have now been recognized and their incidences quantified. Their frequencies differ widely—from 15 million to 10 000 per year, particularly among ethnic groups.

Examples of human diseases and conditions caused by mutations in germ cells

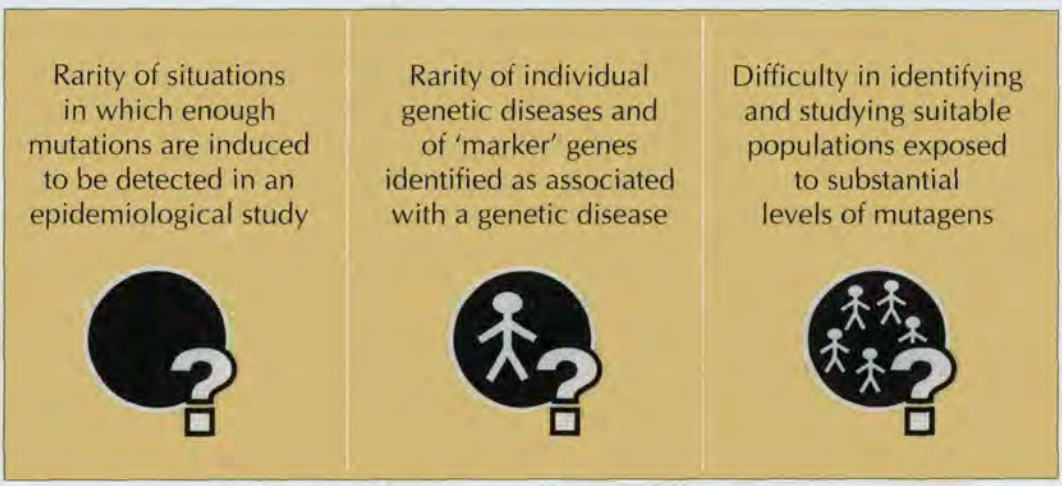
Genetic disease or condition	*Estimated no. of cases in the USA*
Dyslexia	15 000 000
Hardening of arteries	6 700 000
Cancer	5 000 000
Manic depression	2 000 000
Schizophrenia	1 500 000
Juvenile diabetes	1 000 000
Adult polycystic kidney disease	500 000
Familial Alzheimer's disease	250 000
Multiple sclerosis	250 000
AAT deficiency (emphysema)	120 000
Myotonic muscular dystrophy	100 000
Fragile X chromosome syndrome	100 000
Sickle-cell anaemia	65 000
Duchenne's muscular dystrophy	32 000
Cystic fibrosis	30 000
Huntington's disease	25 000
Haemophilia	20 000
Phenylketonuria	16 000
Retinoblastoma (childhood eye cancer)	10 000

This list gives examples only and is not meant to be exhaustive.

These diseases are presently not curable, are occasionally treatable and can be transmitted to future generations. About 5–10 in 100 newborns in the USA have a disease or a genetically determined abnormality. The costs of caring for these children represent a considerable burden on the health services, estimated to range from 25 to 30% of all expenditure on health care.

To date, it has not not been possible to link any human genetic disease with specific mutations induced by radiations or chemicals.

Why we have still not identified human germ-cell mutagens



But the well-documented existence of mutagens that cause germ-cell mutations in non-human species and our knowledge of genetics, mutagenesis and inherited diseases leave no room for doubt that human exposure to germ-cell mutagens entails a risk that a heritable disease will be induced. The identification of germ-cell mutagens and how to manage them are therefore important scientific objectives—not only for human populations but for all the biota that form the complex interrelationships necessary for the continuation of life on earth.

New techniques evolving from research in molecular biology for detecting genetic changes hold promise for studying this problem.

Mutations and Cancer

Mutational damage induced in *somatic cells* can result in effects ranging from cell death, through changes in metabolism and other cell characteristics, to a change in or loss of the natural regulation of cell proliferation. Disruption of normal restraints on growth can lead to uncontrolled multiplication of cells—and therefore to tumours and, ultimately, metastatic cancers. A primary concern with regard to exposure of somatic cells to mutagens is therefore the induction of cancer.

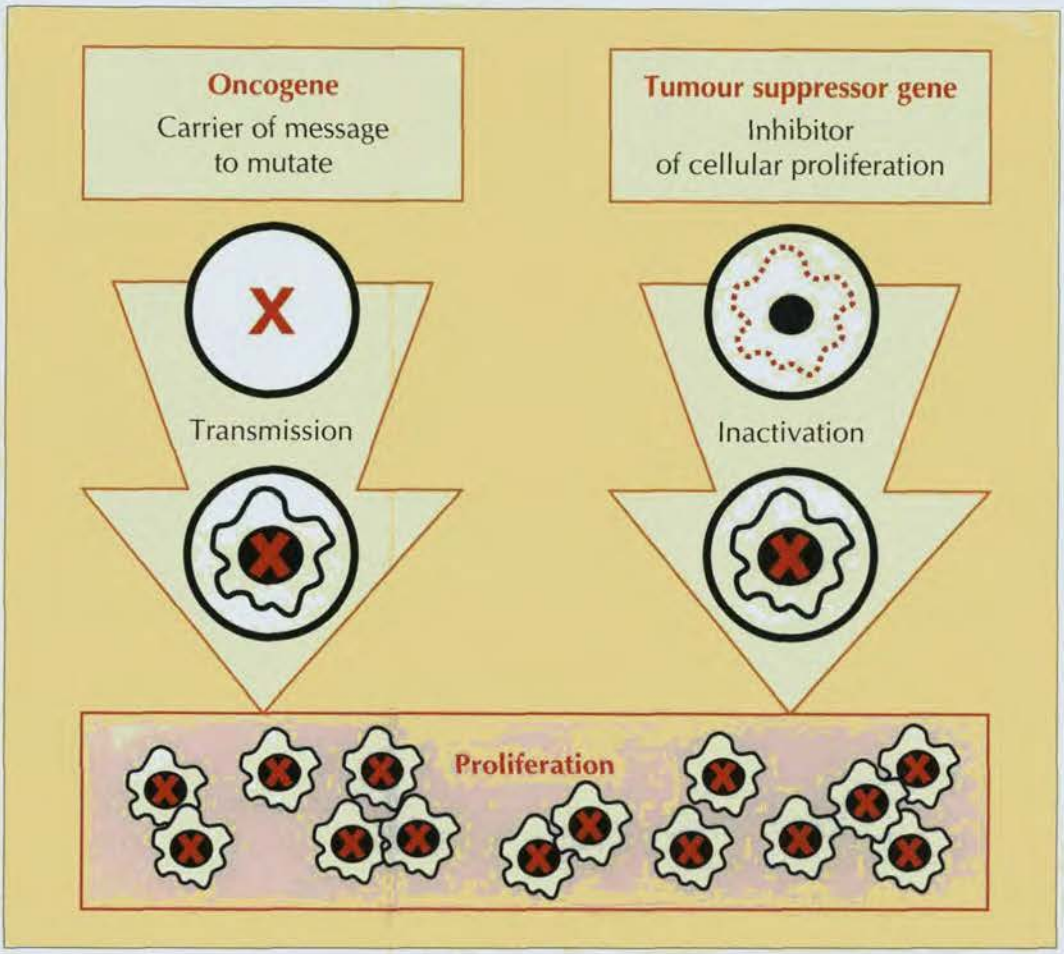
Although our knowledge of the molecular events associated with the induction of cancer is increasing rapidly, the precise mechanisms by which carcinogens induce cancer remain unknown.

Mutation is clearly involved in the process by which some agents act. Recently, specific genes have been discovered that, when mutated, result in cancer. These genes fall into at least two general categories: *oncogenes* and *tumour suppressor genes* (or anti-oncogenes).

Oncogenes are altered forms of normal genes, called proto-oncogenes, many of which are involved in the control of cell growth and differentiation, the process by which the organism increases in organization and complexity during development. Genetic alteration (mutation) of proto-oncogenes can change their function, giving rise to unregulated cell division and, ultimately, cancer.

In contrast to oncogenes, genes have been identified that result in cancer when their function is *lost*. These tumour suppressor genes normally play a role in constraining cell division, so their absence or inactivation by mutational damage allows cell division to get out of hand. Most tumours seem to be due to mutations in several genes, including proto-oncogenes and tumour suppressor genes.

Mutations in somatic cells have also been implicated in other health problems. Mutagens seem to be involved in causing cardiovascular disease, senile cataracts and certain diseases of the digestive tract. The phenomenon of ageing appears to be due in part to mutations.



Risk Assessment

Once a mutagen has been identified, the nature and magnitude of the hazard it presents to the somatic and germ cells of exposed species must be evaluated by the *risk assessment process*. The degree of risk is determined by the rate of exposure to the agent, the ability of the organism to detoxify it, the capacity of the organism to repair its own DNA and the intrinsic potency of the mutagenic agent.

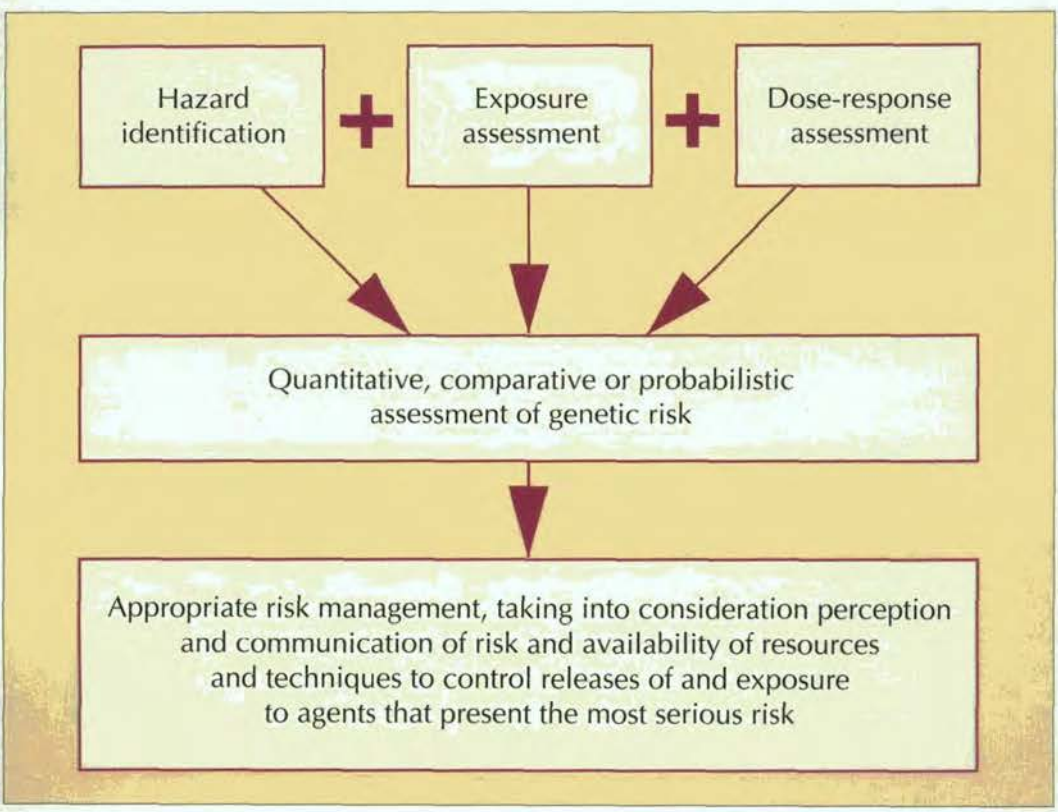
The assessment is usually divided into four stages, as shown in the Figure :

❶ **hazard identification**
—the availability of the agent in the environment and its harmfulness;

❷ **exposure assessment**
—the location of the agent in the environment and its transport and access to the target species;

❸ **dose–response assessment**
—the relationship between dose of the agent and human disease incidence (potency);

❹ **risk assessment**
—assessment of the probability of an increased frequency of disease or death associated with a given exposure.



Genetic risk assessment has not previously been formalized, because of serious gaps in our knowledge of how to characterize risk, of human disease epidemiology, of the comparative sensitivity of the germ line of different species and of how to extrapolate dose–response relationships among species, and because of the tremendous diversity of reproductive processes that is found in the whole range of plant and animal life.

At the request of the United Nations Environment Programme (UNEP), the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) evaluated the state of the art of genetic risk assessment and proposed a set of recommendations for the identification, analysis, assessment and management of risks to humans and other biota associated with exposure to genetically active substances in the environment.

Characterization of Risk for Non-human Biota

Relatively little empirical research has been conducted on genotoxicology in the global ecosystem. Our discussion is thus based largely on theoretical generalizations.

In the context of human health, the concern is primarily *individuals*. In the area of ecological health and human food production, the main focus is generally on *populations*. The degree of concern about effects on non-human biota usually depends on the roles such populations play in the stability of the ecosystem and in agricultural and marine productivity.

Because of this focus on populations, there is little reason for concern about induced *somatic mutations* in non-human biota. Somatic mutations disappear with the death of the individual, so that large populations with high rates of reproduction will not be affected by the premature loss of a limited number of individuals to mutation-induced diseases.

Only in extremely small populations can somatic mutations contribute to extinction. Diseases induced by deleterious somatic mutations can, however, serve as practical biological monitors of pollution.

An increase in the frequency of new *germ-line mutations* with significant effects could impair the health of a population, although the strong selective pressures that normally operate against deviant individuals could reasonably be expected to eliminate such mutations well before they become fixed in the gene pool. A long-term increase in the rate of occurrence of changes in the gene pool would thus have no effect on the population in a stable environment. A new heritable mutation may, however, prove advantageous for the species in which it occurs but disadvantageous to humans—such as resistance to pesticides and herbicides and to antibiotics, and changes in the virulence or in the hosts of a pathogen. In some cases, the genes that control these characteristics may be propagated 'horizontally' from organism to organism or even across 'species boundaries.'

The primary consequences of germ-line mutations in non-human biota are:

Possible reduction of population fitness, with loss of individuals of a species that are no longer able to survive in their normal environment	*Loss of occasional individuals of a species may not present a significant danger to the ecosystem, but a consistent reduction in population fitness could lead ultimately to alterations in the delicate balance required to keep the food chain intact.*
Possible damage to the germlines of crop plants or domesticated animals used as food by human populations	*Such damage is unlikely to raise problems for human populations, as methods are available for continuing to select for and improve existing germ-lines.*
Alterations to the genomes of pathogenic organisms in the ecosystem that increase their virulence, range of hosts or resistance to antibiotics	*Such alterations might affect the health and well-being of both humans and economically important domesticated species.*

Our knowledge of the ecological consequences of increased mutation rates in wild populations of non-human biota or of the appearance of new mutant organisms in the ecosystem is extremely limited. While it is reasonable to wish to reduce exposure to mutagens across all life forms, the benefits relative to the costs of controlling exposures may not warrant the allocation of substantial resources to the assessment of risks to non-human biota. Ecological risks are best assessed on a case-by-case basis. The diversity among living species of metabolic and reproductive characteristics and of the mechanisms of DNA repair makes it impossible to gather enough information to attempt a quantitative assessment of the risk posed by a mutagen, in all but a very few instances. Accordingly, a conservative approach to these issues is the wisest.

Hazard Identification

This step in the risk assessment process often begins with a listing of agents considered to be potentially genotoxic on the basis of :

- their structural relationship to other chemicals known to be genotoxic—structure–activity relationships
- their activity in short-term tests for genotoxicity
- the results of monitoring of humans and the environment
- gross estimates of exposure

Hazard identification is also important in determining whether there is sufficient evidence for proceeding to assessments of exposure and dose–response, or whether more research is needed. Although in most industrialized countries new chemical agents are now screened for genotoxicity before being introduced into commerce, many agents introduced in those countries before 1980 and elsewhere in the world have as yet escaped screening. In addition, very few of the many environmental chemicals that result from combustion, industrial processes, natural processes and natural plant and fungal products have been characterized for genotoxicity.

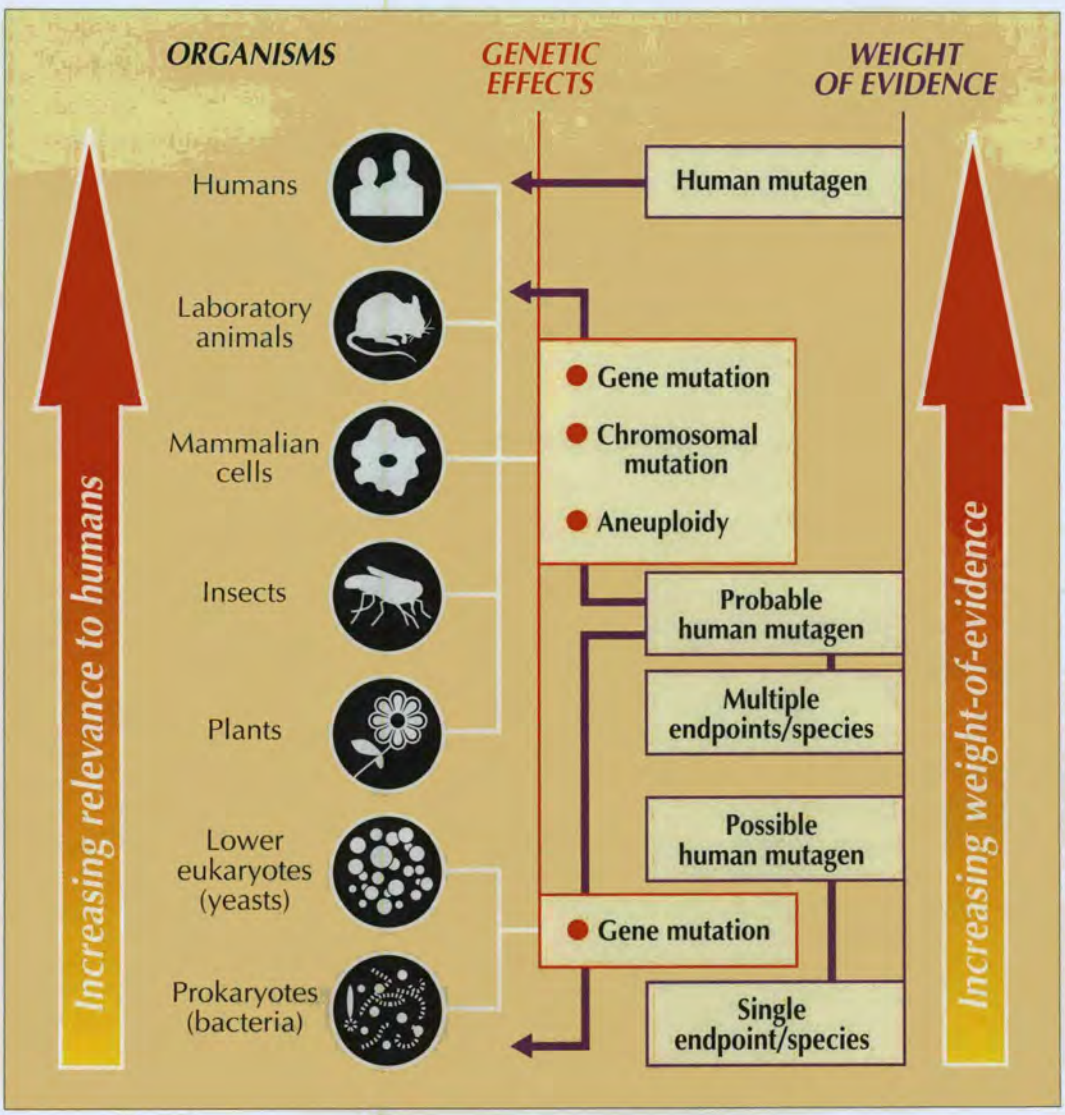
Identification of Mutagens

Uniform criteria do not exist for dividing agents into mutagens and non-mutagens. The criteria used in this document for identifying an agent as a mutagen include a demonstration that it interacts chemically or physically with DNA to induce a change in the sequence of DNA bases or alter the physical structure of chromosomes.

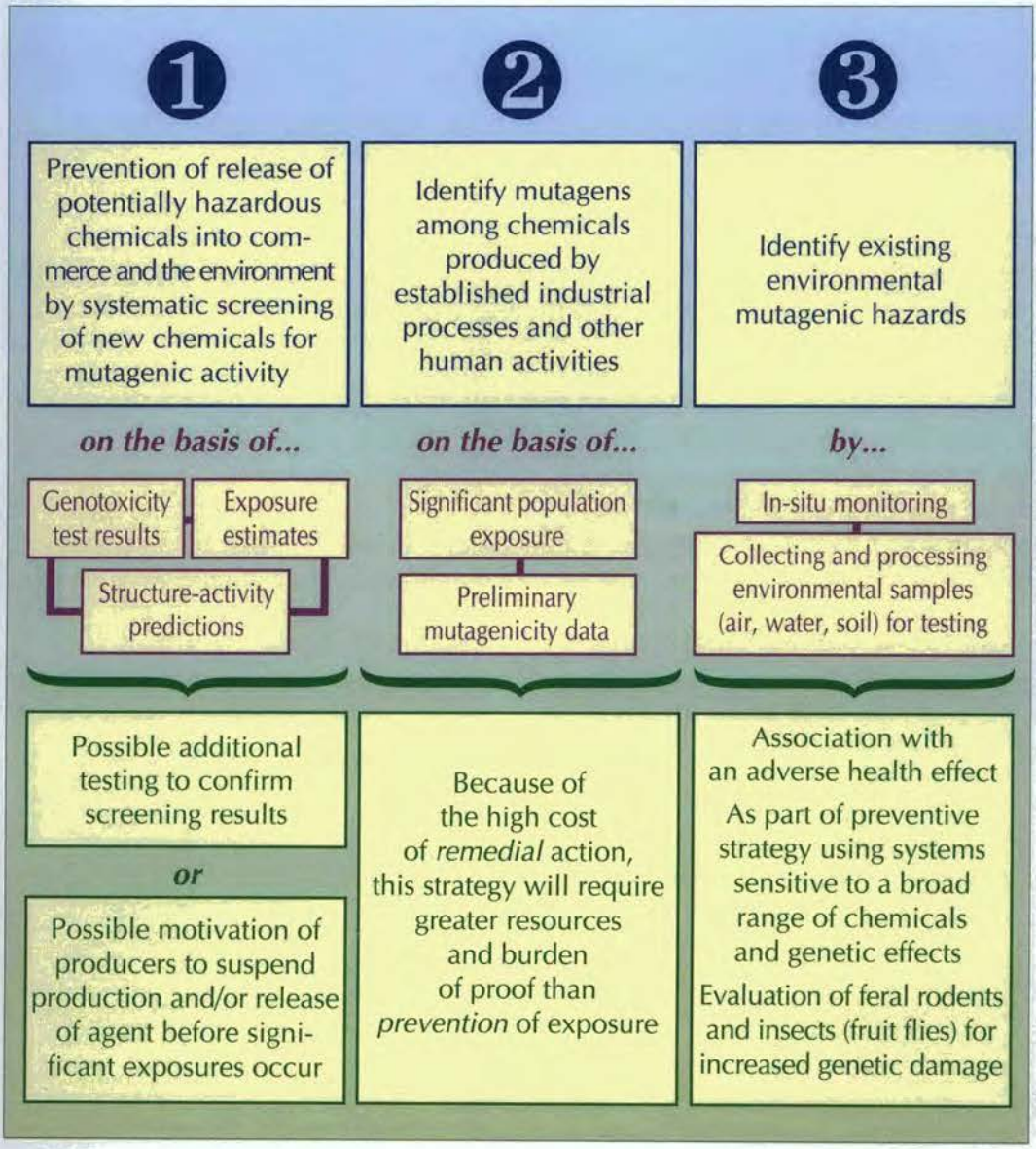
Agents are also sometimes labelled as 'genotoxic'. This term is broader and comprises both mutations and the full range of related events associated with damage to DNA. More than 150 assay systems have been developed to detect these changes, using the full spectrum of organisms, from bacteria to human cells and intact experimental animals; no one test can detect all of them.

Categories of genotoxicity identified in different tests

- Alteration of the sequence of DNA bases
- Alteration of the integrity of DNA
- Exchange or rearrangement of genes between pairs of chromosomes
- Alteration in the separation of maternally and paternally inherited chromosomes during development
- Alteration of the integrity of chromosomes



Classification of genotoxicants by the nature of the genetic lesion they induce is important, because it conveys information about the intrinsic degree of hazard. Identification of the full range of mutagens to which humans and other biota are exposed would be an enormous task, even if the exercise were restricted to chemicals generated by human activities. The process of identification must therefore be selective and depends on the source of the chemical and the nature of the concern.



To characterize the degree of concern about the hazard represented by a particular agent and to determine the associated risks for humans, agents can be classified as:

- **Possible human mutagen**: demonstrated to induce gene or chromosomal mutation in any organism
- **Probable human mutagen**: demonstrated to induce gene or chromosomal mutation in organisms closely related to humans (e.g., rodents); or demonstrated to induce genotoxic effects in many species of organisms
- **Human mutagen**: demonstrated to induce gene or chromosomal mutation in humans. No human heritable mutagens have been identified, but agents are known that induce somatic-cell gene mutations and chromosomal anomalies in lymphocytes and chromosomal aberrations in sperm of exposed humans.

Tests have been developed to detect a wide variety of genetic damage. Because such tests take much less time than conventional bioassays using rodents, they have been called 'short-term tests'. The wide variety of end-points and the limitations to different test systems mean that no single test is available that can detect all types of damage. Various schemes have therefore been developed over the years for using a minimal number of tests in combination, to identify an agent as a mutagen or a non-mutagen.

Tests are applied in either sequential (tier) or cross-sectional (battery) approaches. The battery approach, which requires more initial resources than the tier method but less testing time and better use of available technology, is now most commonly used. It is generally agreed that batteries should include tests for detecting the two main classes of genetic damage—gene and chromosomal mutations. Aneuploidy is important, especially with regard to cancer; but there is as yet no simple, reliable assay that can be used routinely to detect it.

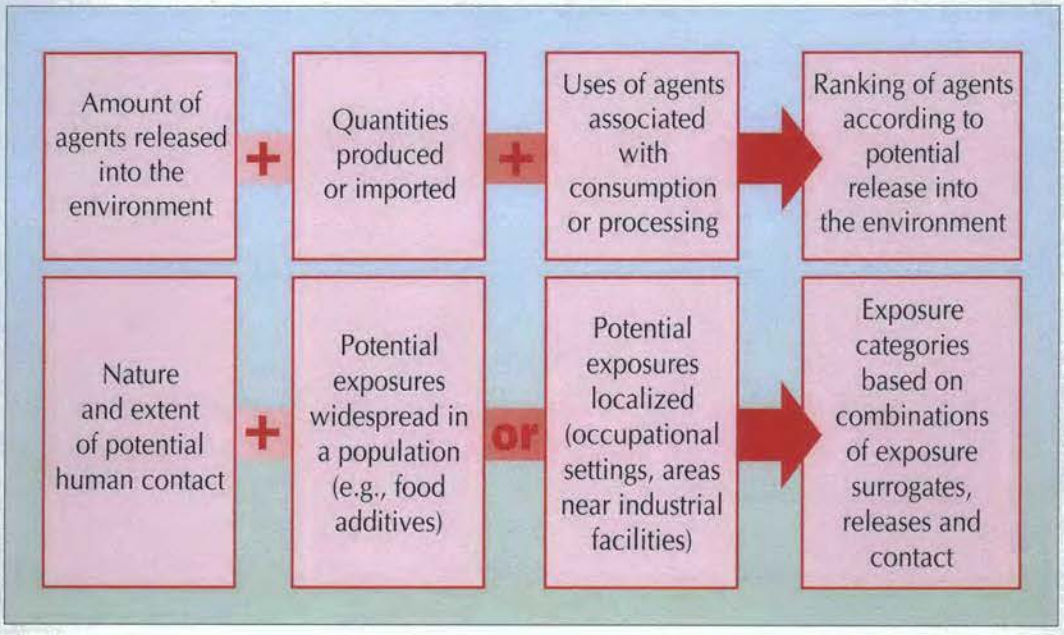
The **tier** approach, with tests applied sequentially	➡ Inexpensive, rapid tests conducted *in vitro* erring on the side of false-positive results	➡ A single response can determine a genetic hazard
The **battery** approach, with several tests applied in parallel	➡ Each test selected to compensate for limitations of other tests in battery	➡ A single response not usually sole determinant of genetic hazard

Exposure Assessment

Qualitative Exposure Assessment

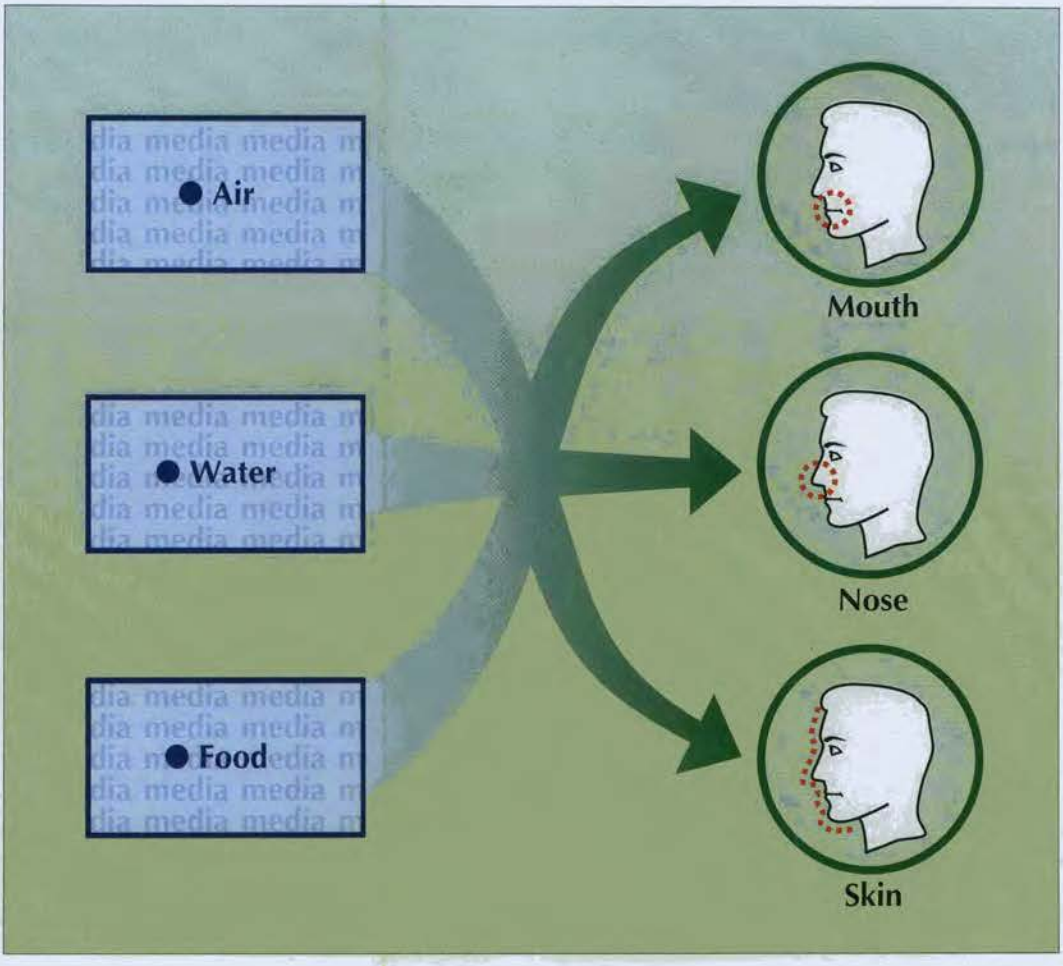
Assessments of the potential magnitude of human contact with a given genotoxic substance may rely on surrogate measurements of exposure:

Components of qualitative exposure assessment



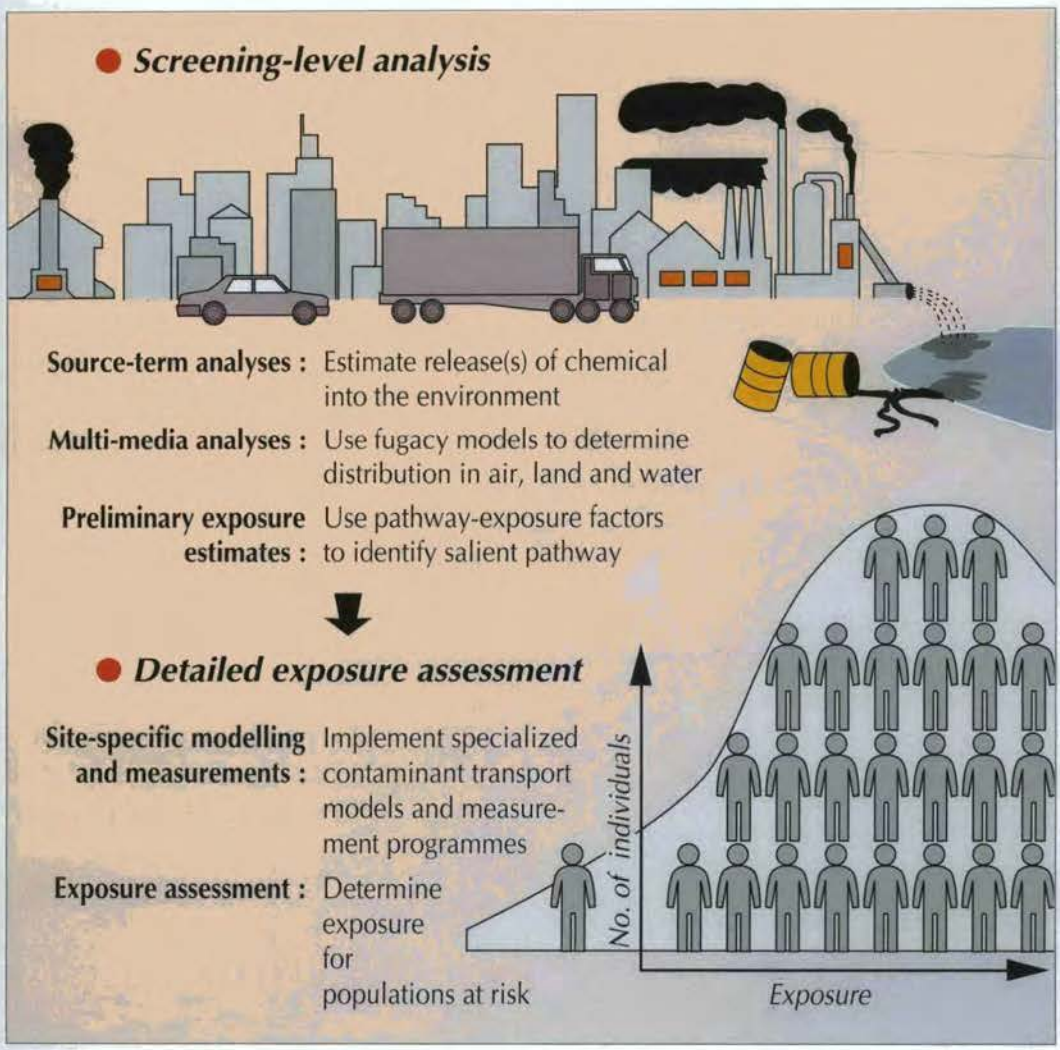
Exposure may occur *via* various routes (mouth, nose, skin) and various media (air, water, food). If all possible pathways cannot be assessed, the most important must be identified. By identifying the sources of the agent and estimating the rates of its emission or release, modelling can be combined with estimates of the concentrations of the agent in important compartments such as air, water and food.

It is useful to be aware of the nature of the adverse effect. If the agent has chronic effects, assessment of the average annual exposure may be adequate; if the effect is focused on a critical period of exposure (e.g., during one stage of reproduction), either the peak exposure or total exposure during that period will be necessary to link the exposure assessment to the dose–response data.

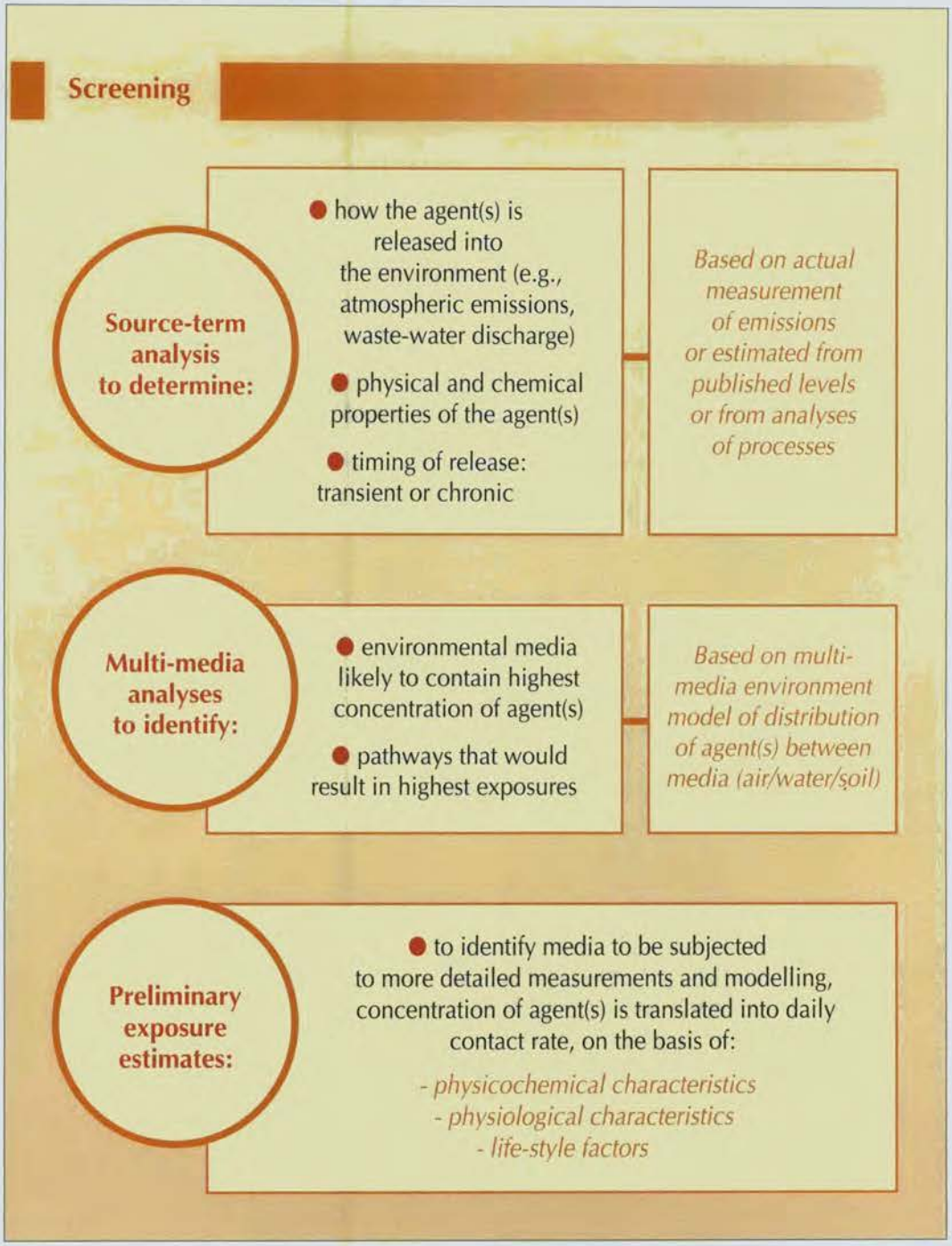


Quantitative Exposure Assessment

The basic objective is to determine, in a cost-effective manner, the concentrations of a genotoxic agent in media with which humans come into contact, such as air, water, soil, food and beverages, and the rates of contact with those media. Assessment strategies should be focused on the most important pathways of human exposure.



Quantitative exposure assessment involves both screening and detailed determinations of the components of the exposure.



Several steps are involved in the detailed exposure assessment.

Detailed exposure assessment

Determine spatial and temporal variations in concentration of agent(s) in air, water, soil and biota

- 1 - Site-specific modelling and measurement:

● for annual average concentration of an airborne substance, e.g., around an industrial facility	*Seasonal meteorological data on wind speeds, mixing height and atmospheric stability*
● for annual concentrations at fixed locations, e.g., downwind from a facility	*Monitoring equipment operated long enough: particulate matter analysed in laboratory*
● for climates where people spend much of their time inside	*Determination of agent(s) indoors*
● for concentrations in groundwater	*Hydrological properties of aquifer, by installing sample wells*

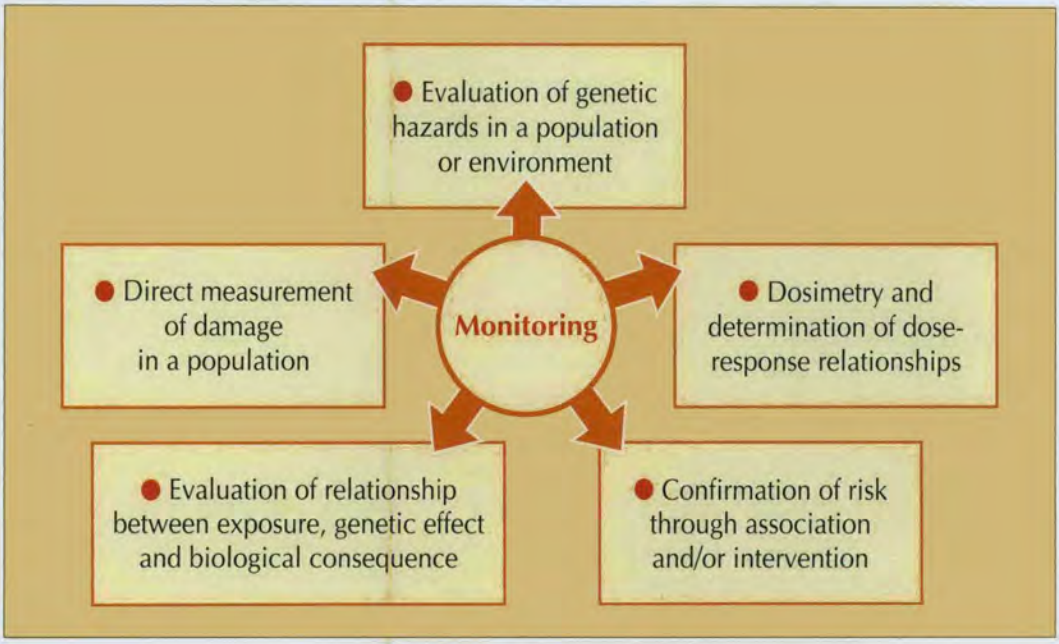
- 2 - Exposure assessment:

Population exposures to an agent =
no. of individuals exposed to a medium
x *concentration of agent in medium.*

Although exposure assessments result in estimates of the rates of contact with an agent, the predicted results are usually affected by various uncertainties. An uncertainty analysis should therefore be done—however rudimentary — to determine the assumptions, distributions of parameters and other variables involved.

Monitoring

Monitoring of the environment or of the population at risk can be involved at each stage of risk assessment to achieve different objectives:

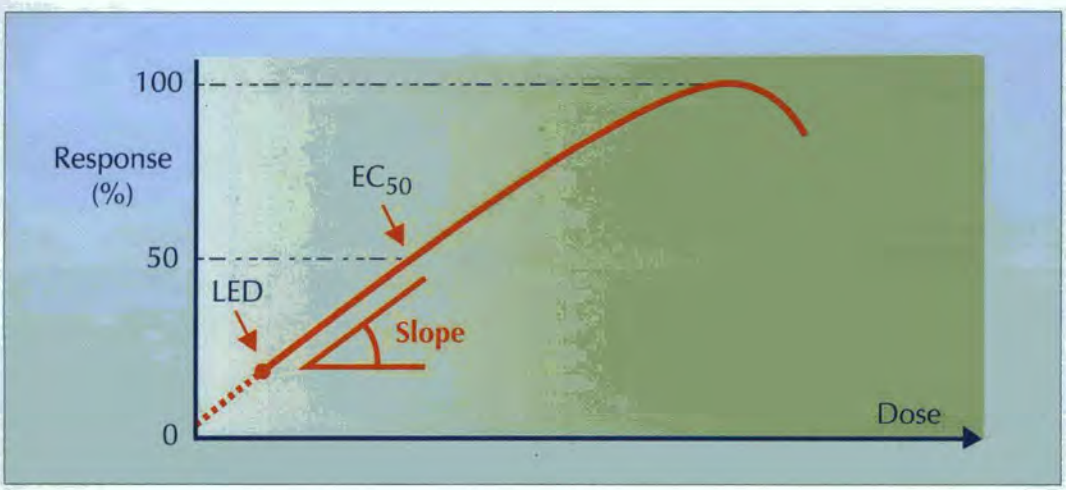


Dose–response Assessment

Assessments of dose–response involve quantification of the potency with which an agent induces genetic damage of direct relevance to the species at risk. Thus, can be assessed by assaying human risk is to assay somatic- or germ-cell mutations in a mammal, such as a mouse or a rat.

In dose–response assessment, the measure of the dose is usually that applied to the test system. Increasing efforts are being made, however, to determine the concentration of the agent that reaches the target tissue or molecule and the time it stays there. Ideally, data on dose–response should be available from studies of humans or of mammals *in vivo*, so that extrapolations can be made from the high doses used in test systems to the low doses usually encountered in the environment or from results in animals to the human situation. Such data are not often available.

Some approaches to determining mutagenic potency



Two approaches can be used to determine mutagenic potency: using the slope of the dose–response curve (i.e., per cent response per increment of dose tested) or using the dose at which a specific response is obtained, such as the lowest effective dose (LED) tested or the dose at which a 50% response is obtained (50% effective dose, EC_{50}).

Use of the slope method is limited by the difficulty in extrapolating down to the low dose levels usually encountered in the environment. The advantage of using the dose-specific methods is that data from several different calculations can be readily compared or combined.

Genetic Risk Assessment

The diversity of possible adverse effects that may result from exposure to environmental genotoxic pollutants requires the introduction of special strategies into the basic elements of risk assessment.

When information is limited or when only a general characterization of risk is required, a *qualitative assessment* can be made. But in exceptional cases, when the available information includes evidence found in humans or data obtained in mammals that is of direct relevance to humans, risk assessment can be *quantitative* or *comparative* and can be expressed as a *probabilistic* risk of mutation occurring in humans.

Merits of three strategies for risk assessment

Qualitative assessment	Comparative assessment	Probabilistic risk assessment of a single agent or exposure
Serves as a guide for further study of an agent and as an alert	Establishes priorities for genotoxic risks	Allows an estimate of the probability of a change in mutation rate

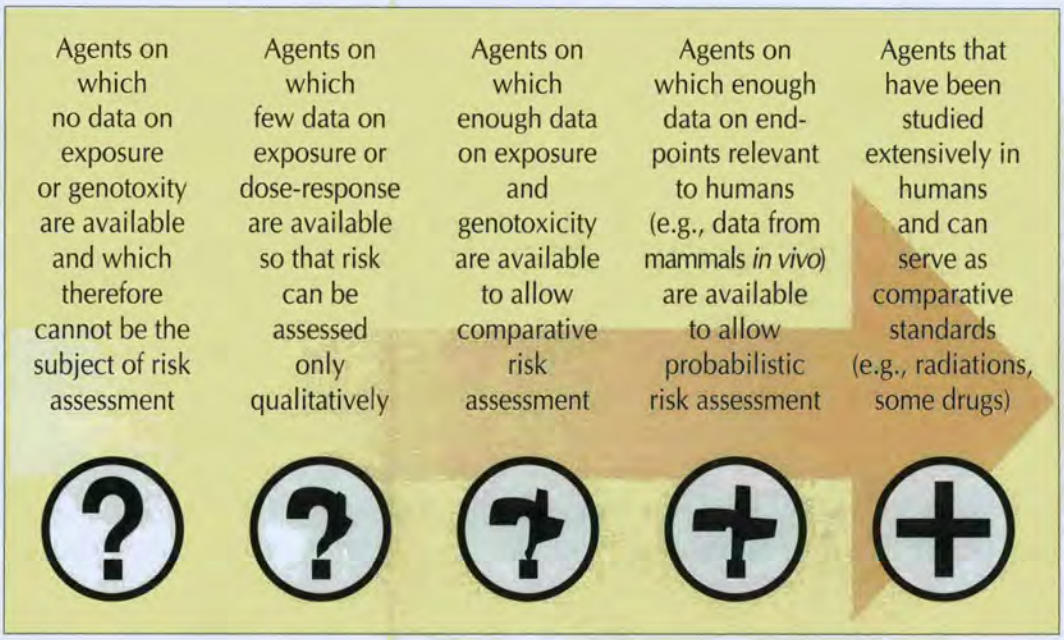
In comparative risk assessment, agents are compared with respect to their potency in inducing genotoxic effects. A probabilistic risk assessment can be made only when a dose–response relationship is known, so that a quantitative estimate can be made of the probability that a change in mutation rate will occur.

Choice of Risk Assessment Strategy

Multiplying the assessments of exposure and dose–response gives the risk or probability of an adverse outcome. This stage may also involve interpretation of the societal or biological implications of the risk. Because mutation is not itself a disease, assessments of genetic risk necessarily lead to estimates of the risk that the genetic damage will result in disease or death. The clearest illustration of effect would be an alteration in the incidence of the genetically related condition; but, since mutations are so rare, it will seldom be possible to measure their effects directly.

The *availability of data* on the genotoxicity of and exposure to an agent is the first factor to be taken into account in determining which strategy to use in characterizing risk. All agents—chemicals, radiation, mixtures, sources of pollution—can be classified on the basis of this factor.

Classification of agents by data availability



The largest number of agents is found in the first group, the numbers decreasing dramatically by the fourth or fifth. The emphasis in practice is therefore on developing strategies to characterize the risk presented by agents in the second and third groups.

The *purpose of the final risk assessment* is the second factor that influences the choice of strategy for preventing disease. If the purpose is purely scientific—e.g., as an exercise for improving methods of assessing risk—the choice of the strategy will be dictated by the scientific issue being addressed. Most frequently, the purpose is to protect human health. Scientific attention has been paid primarily to improving methods and reducing the uncertainties of probabilistic risk assessments. Comparative methods may provide a more rapid and practical approach in many situations. Probabilistic assessment methods are necessary, however, when there is heavy or widespread human exposure to an agent.

The exercise of risk assessment should result in clearly organized, clearly presented scientific information about public health hazards, to provide a solid scientific basis for policy decisions (risk management).

Clear understanding of the risk management process should therefore help to establish the objectives of the risk assessment, which can range from very qualitative rankings of risk to highly quantitative information on safe levels of exposure to an agent.

Risk assessment strategies to meet different risk management objectives

Risk management objective	Assessment strategy
Ranking of genotoxic risks of various environmental media (air, water, soil, waste) to establish priorities for research funding	Qualitative or comparative
Ranking of sources of environmental pollution (energy production, chemical industries, agricultural run-off) in a geographical region to decide on allocation of funds for pollution control	Comparative
Assessing the risks of all sources of air pollution in a geographical region to determine which contribute most to genotoxic risk, with a view to initiating and/or evaluating regulations on air pollution	Comparative
Assessing the genotoxic risks presented by different techniques for water purification (e.g., chlorination, ozonation), with a view to setting specific emission standards for each technique	Probabilistic
Assessing the genotoxic risk presented by allowing a food additive to be used in baby cereals, soft drinks and other food products	Probabilistic
Assessing waste chemicals to determine whether they should be classified as potentially hazardous and therefore not be mixed with normal municipal waste	Qualitative
Assessing new chemicals before their introduction into commerce to determine whether they should be allowed without regulation, regulated or prohibited	Qualitative, possibly followed by probabilistic if regulation required

Strategies for Qualitative Risk Assessment

For those agents for which there are so few data on exposure and/or dose–response that only a qualitative characterization of risk can be consi dered, a simple, graded classification of genotoxic potency will suffice for making a rough, qualitative risk assessment.

As few as two or three categories of genotoxicity and prevalence in the environment can provide three qualitative levels of risk—high, medium and low.

The refinement of the classification will clearly determine the degree to which the qualitative assessment can be used. Both the qualitative exposure assessment and the genetic potency must be taken into account.

The qualitative approach has long been used to generate risk assessment. Groups of experts evaluate the weight of the evidence that an agent presents a human health hazard and judge the level of exposure at which an effect might occur. Identification of the nature of the genetic effect does not provide information about the degree of hazard, but it does indicate the nature of the hazard and, even more important, the nature of the evidence.

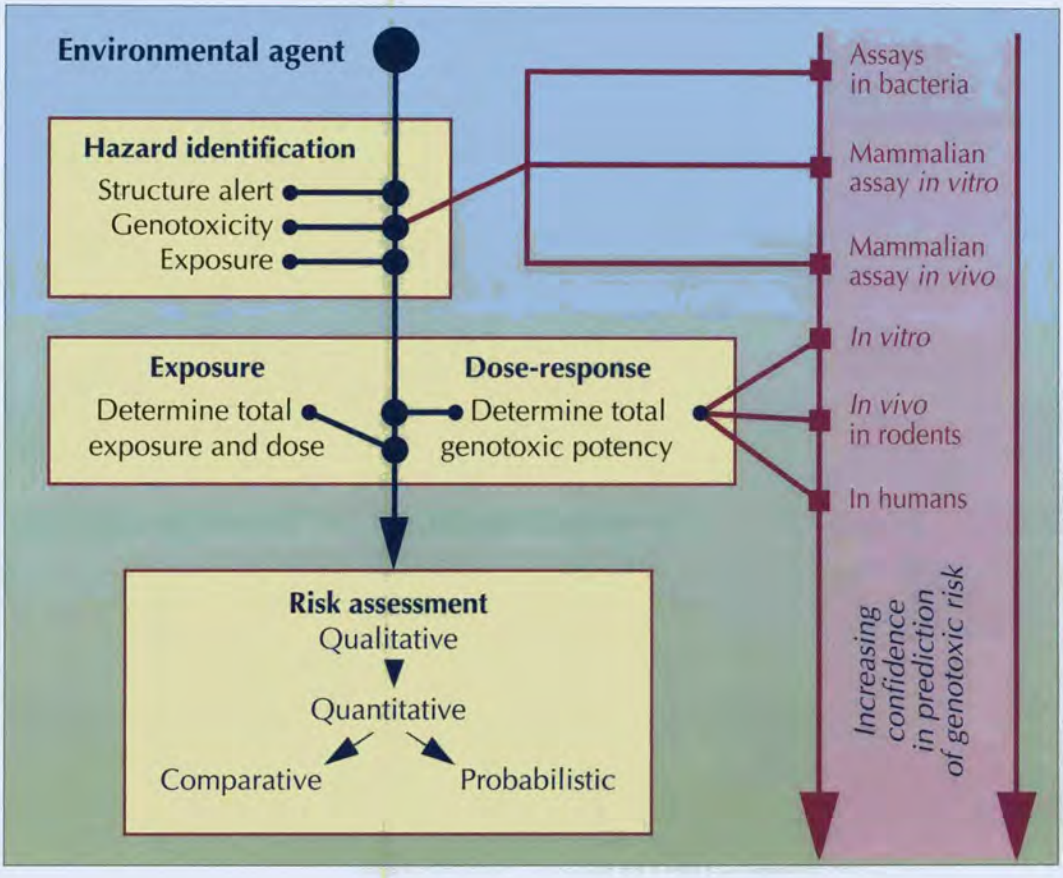
Schemes based on determinations of the weight of the evidence have been developed for classifying genotoxic agents that may induce heritable genetic damage.

A rare, synthetic chemical which is not found in the environment but has been shown to be a mutagen in many test systems would be rated as less high a risk than a chemical to which people are widely exposed and which has been tested for genetic activity only in microbes.

Such assessments can help to establish the order in which agents should receive attention—for further measurements or definitive action.

Strategies for Quantitative Risk Assessment

Quantitative estimations performed to date of genetic risks and of the increased incidence of genetic disease that will be caused by exposure to mutagens have concentrated primarily on the production and transmission of germ-cell mutations. Furthermore, risks for germ-cell mutations have been estimated in relation to ionizing radiation but to a much lesser extent in relation to chemicals. Even when good evidence is available from experimental situations, it is difficult to extrapolate from mutation incidence in rodents to predict that in humans, owing to our current lack of information on the association between heritable damage in rodent germ cells and the incidence of human disease.



Quantitative risk assessment combines the extrapolated estimate of potency with the estimates of individual and population exposure to determine either the probable or comparative risk. Risk can be estimated for individuals or for populations.

Extrapolations from the high doses used in experimental studies to the low doses usually encountered in the environment and extrapolations of results obtained in studies of experimental animals to the human situation remain the two most controversial issues in quantitative risk assessment. Various approaches to extrapolation are available, which range from definite, probabilistic methods to methods that provide a relative ranking of genetic risk.

The levels of chemicals to which humans are exposed, and the doses that actually reach the target site in the body, are generally much lower than the doses tested in laboratory animals.

The model used most commonly to extrapolate to low doses is the assumption that even low doses will have some effect, i.e., that there is no dose below which no effect will occur (non-threshold). For cancer risk assessment, newly proposed, biologically based model of dose–response, entailing an initial reaction of genotoxic chemicals with DNA, followed by mutations, cell death, cell growth and proliferation, are expected to become increasingly important. Their use will call for additional data on biological dosimetry and molecular mechanisms.

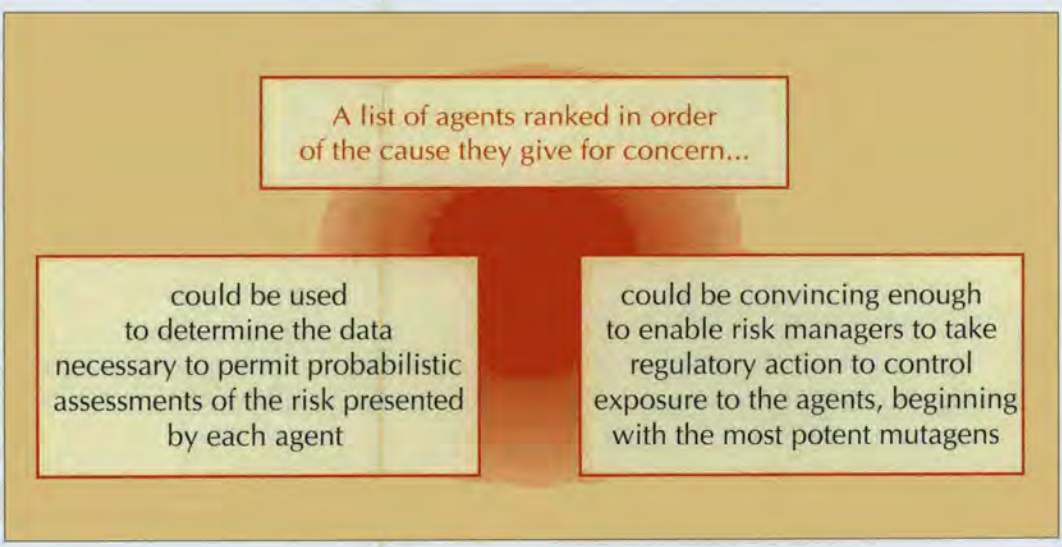
The difficulties in extrapolating results obtained in laboratory animals to humans are most serious when assessing the probability that an inherited human disease was caused by exposure of one or both parents to genotoxic agents. Even if the best possible experimental data are used—observations of mutagenicity in the germ cells of rodents—extrapolation to disease incidence in humans is problematical, because of lack of knowledge about the fraction of heritable mutations that results in human disease and the sensitivity of different species to the agent.

In principle, all agents that have undergone quantitative risk assessment should be comparable. With increased understanding of the significance to health of changes in mutation rates, risks determined in this manner should be comparable to other types of risk, as from fire, road accidents and infectious diseases.

A further step would be to reduce all such risks to a common denominator—making them comparable to other societal options, such as the economic benefits to be derived from use of the agent. Despite the practical problems likely to be encountered—particularly the scarcity of the necessary data—such comparative risk assessments might be attempted, if only to gain better understanding of the limitations of less rigorous approaches and to encourage the collection of information.

All comparative methods are based on comparing the relative potency of the agent to that of a standard agent, such as radiation or a well-studied chemical. The underlying assumption is that the relative potency in humans is equivalent to or may be predicted from the potency observed in a bioassay.

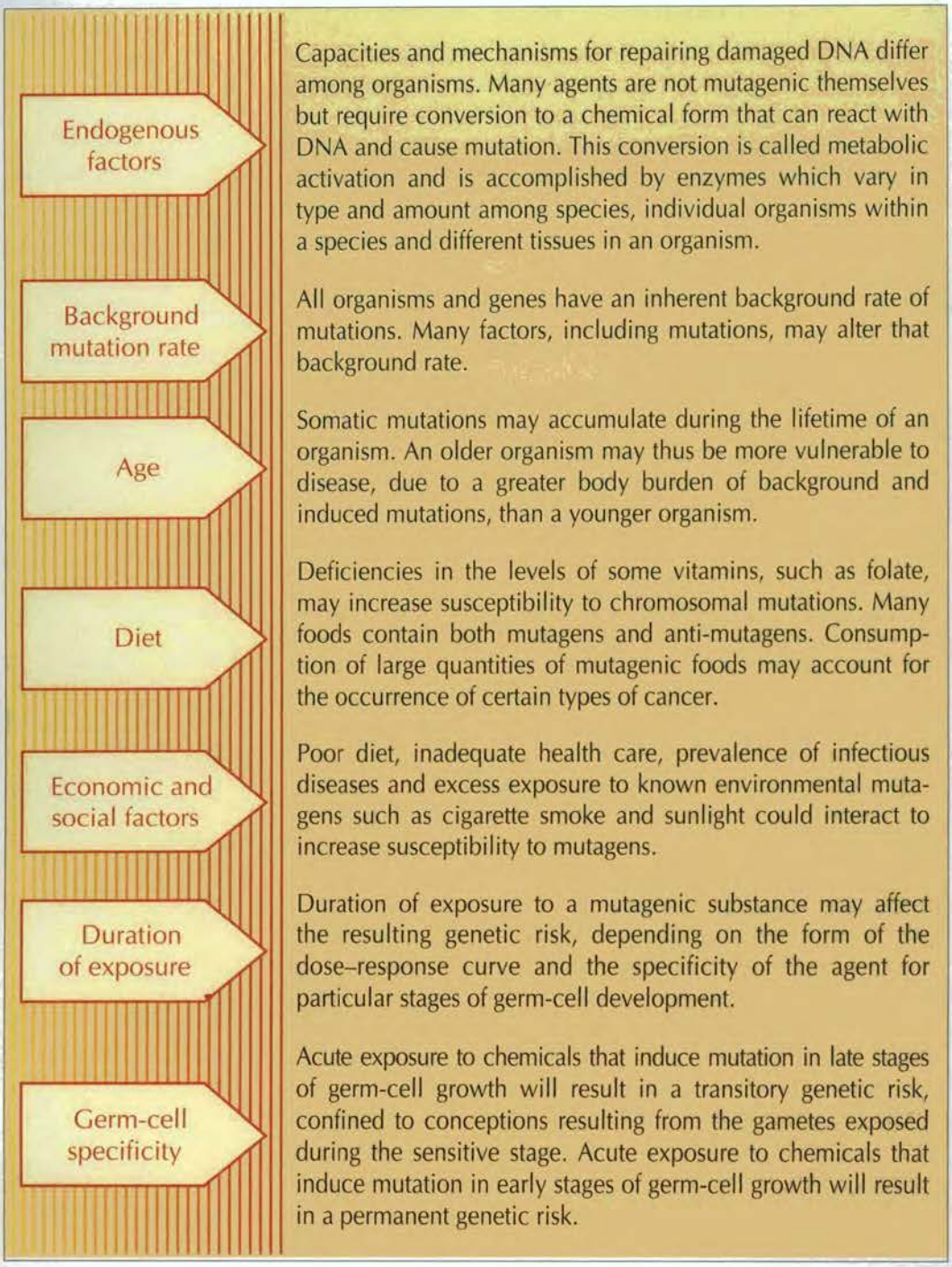
Possible uses of comparative risk assessments



Factors that Affect Risk

Risk can be assessed quantitatively only if account is taken of other factors that might change that risk.

Some examples are:



Capacities and mechanisms for repairing damaged DNA differ among organisms. Many agents are not mutagenic themselves but require conversion to a chemical form that can react with DNA and cause mutation. This conversion is called metabolic activation and is accomplished by enzymes which vary in type and amount among species, individual organisms within a species and different tissues in an organism.

All organisms and genes have an inherent background rate of mutations. Many factors, including mutations, may alter that background rate.

Somatic mutations may accumulate during the lifetime of an organism. An older organism may thus be more vulnerable to disease, due to a greater body burden of background and induced mutations, than a younger organism.

Deficiencies in the levels of some vitamins, such as folate, may increase susceptibility to chromosomal mutations. Many foods contain both mutagens and anti-mutagens. Consumption of large quantities of mutagenic foods may account for the occurrence of certain types of cancer.

Poor diet, inadequate health care, prevalence of infectious diseases and excess exposure to known environmental mutagens such as cigarette smoke and sunlight could interact to increase susceptibility to mutagens.

Duration of exposure to a mutagenic substance may affect the resulting genetic risk, depending on the form of the dose–response curve and the specificity of the agent for particular stages of germ-cell development.

Acute exposure to chemicals that induce mutation in late stages of germ-cell growth will result in a transitory genetic risk, confined to conceptions resulting from the gametes exposed during the sensitive stage. Acute exposure to chemicals that induce mutation in early stages of germ-cell growth will result in a permanent genetic risk.

Applications of Comparative and Probabilistic Risk Assessments

Components of the general framework for comparative risk assessment are used in many countries to regulate whether new chemicals will be allowed into commerce and to set acceptable limits on exposure to chemicals. Quantitative risk assessment has been used most widely to estimate human cancer risks. A generic application of the risk assessment framework has been described, beginning with hazard identification and progressing to risk characterization estimated as the relative or comparative risk of exposure to genotoxic agents.

Possible applications of risk assessment methods are:

- Ranking the potential hazard of new chemicals to limit the introduction of hazardous chemicals into commerce, the environment, food and the workplace.
- Ranking emissions from urban, rural, industrial and energy-generating activities to determine the emissions that present the highest potential genetic risk.
- Ranking genetic risks in geographical areas to set priorities for intervening in and controlling the release of genotoxic agents.

Risk Management

Risk Communication

Communicating the results of risk assessments effectively to risk managers and to members of the public is an important aspect of risk management. Failure to communicate the results properly can lead to:

- incorrect perceptions of the nature or magnitude of the predicted risks
- inappropriate decisions about managing the risk

Such outcomes are often unexpected by risk assessors who presume that their estimates and supporting analyses are sufficient to justify subsequent actions or provide the necessary information. Risk communication is not a one-way process between the risk assessor and the user but a two-way process of exchange of information.

When the public perceives itself as being directly involved in managing the risk that has been assessed, the credibility and neutrality of the organization that assesses the risk can influence critically the transfer of information on risk assessments and its interpretation. Neutrality can be compromised if the assessor uses comparisons designed to increase the acceptability of the risk. The acceptability of the risk of a given toxic substance is a complex function of perception, cultural factors, economic considerations, comparisons with other risks and levels of knowledge and education. Issues pertaining to risk acceptance are best left to risk managers.

When the public is intimately involved in the risk management process, the risk assessor should determine the level of information that is needed. It may be necessary to define in more detail certain assumptions and parameters. Additional

discussion may be required about the inferences, uncertainties and limitations of the analysis. Meetings can be held for exchanges of information between the assessor, the media and the public. These are most helpful and constructive when held early in the risk assessment process—not after the assessment has been completed.

Strategies for communicating risk are important. It may even be prudent to develop communication strategies at the same time as the risk assessment approaches are being planned.

Control Measures

In rare situations, the recognition of a genetic hazard can provoke *direct measures* for control. A more common sequence of events is as follows :

- recognition of a hazard
- estimation of risk in the expected exposure situation
- initiation of appropriate control measures to reduce the risk to acceptable levels

Selection of control measures in a given situation is influenced by many subjective and often competing factors, and specific measures cannot be recommended. Risk estimation usually involves calculation of the projected incidence of a genetic disease in a particular population. The projected incidence will be for a given time frame, which itself is determined by the disease produced. For example, exposure to a leukaemogen results in the induction of leukaemia within about 10 years; while bladder carcinogens usually result in a tumour about 20 years after exposure.

Subjective elements

The interplay of subjective and objective components of risk management can be illustrated as follows :

- an incidence of 5 in 10^4 cases of skin cancer among 50 miners exposed to white arsenic
- an incidence of 5 in 10^4 cases of leukaemia among 2 million people exposed to an uncontrolled source of radiation

These two objective risk estimations will be influenced by a range of subjective influences, which will eventually affect which control measures are instituted.

The first subjective influence is the extent to which the disease is encountered in reality. Surveillance of 50 miners is unlikely to yield cases of skin cancer, while leukaemia will be perceived as commonplace among a population exposed to radiation. The expected frequencies in these two situations will be 0.25 cases of skin cancer and 1000 cases of leukaemia, even though the individual risk of getting cancer is identical in the two situations.

The perception of an induced disease is further subject to the influence of whose responsibility it is to institute control measures. For example, a government authority would probably choose to sponsor measures to control the source of radiation, while the mine owner would be faced with the single alternative of improving hygiene in the mine.

A further subjective complication is introduced by the concept of risk–benefit analysis. The basic problem is that the risk may accrue to different individuals or groups of people from those who receive the benefit. When different groups are involved, the possibility of legal intervention gives rise to concepts such as 'negligence', 'acceptable risk' and 'relative risk'. These terms are of obvious relevance to control measures, but they are subjective and derive from socioeconomic considerations.

When the risk and the benefit accrue to the same person or group, the concept of personal freedom intervenes. Thus, a community of people may expend great effort in measuring minute levels of a pesticide in their environment while accepting the personal freedom to smoke tobacco. The current interest in passive smoking illustrates how small changes in perception of risk can have profound effects on risk management—control measures are instituted for the lesser hazard of passive smoking, leaving the major hazard of tobacco smoking uncontrolled.

The final subjective component of risk management is the extent to which institution of the control measures is practicable. This ranges from the simple decision to stop development of a potential drug found to be mutagenic and for which adequate non-mutagenic analogues exist, to the problems raised by finding that a natural constituent of a staple diet is mutagenic. Two similar risk estimations can lead to different control measures.

In countries still concerned with improving life expectancy or reducing famine, more complex risk management decisions have to be made. For example, a pesticide may dramatically enhance yields of a subsistence crop and thereby save, say, 10^5 lives. The same pesticide may be shown by risk assessment to entail a cancer risk of 1 in 10^5 when present as a residue in the crop. In a developed country, a cancer risk of this level might be considered unacceptable, but when the 10^5 people at risk have actually been kept alive by the crop, a decision is less easy to take.

An example of how to manage risk is provided by occupational exposures, because, in this situation,

- the population at risk is known precisely
- hazardous agents can be identified and monitored
- a wide range of protective measures can be taken
- the efficacy of control measures can be monitored.

The risks to the general population are less easy to manage.

Control of chemical agents from different sources

Source	Exposures	Examples of control measures
Chemicals now in commerce	Manufacture	Reduction of fugitive releases
	Transport	Training of transporters
	Use	Training of users
	Disposal	Stringent regulations in effect in many countries; actions to prevent effluents in groundwater taking into account changes in site integrity
Derivatives of chemical reactions	Combustion	Emission control systems for vehicles, fossil-fuelled electric power plants and waste incineration facilities
Mutagens and carcinogens in food	Natural materials and those produced by cooking	Change dietary and cooking habits

The best way to reduce exposure to mutagens is obviously to prevent them from entering the environment. In many industrialized countries, existing regulatory procedures prevent exposure of their populations to new chemicals that could result in a health hazard. In less developed countries, however, the social priorities may be different.

Expansion of pre-manufacture and pre-marketing testing and regulations could help to reduce exposures to chemical mutagens. Information on the health effects of chemicals already available in commerce and industry and on the testing and regulation of chemicals is given in numerous international publications.

Conclusions

EXPOSURE TO MUTAGENS SHOULD BE CONTROLLED

There are several reasons for attesting to the fact that mutagenic agents are hazardous and that their presence in the environment should be restricted or controlled to the greatest extent feasible.

There is sufficient similarity between the germ-cells of rodents and of humans to assume that human germ-cells can be mutated by chemicals and radiation. In addition, the association between somatic-cell mutagens and germ-cell mutagens in mammals is sufficient to argue that events known to induce damage in genes or chromosomes in human somatic cells may also damage a portion of the germ-cells. Although the quantitative association between mutations in these two cell populations is unknown for most species, this association would be specific for each agent, and it cannot be expressed as a constant.

The relationship between mutations induced in mammalian somatic cells and the development of cancer has been well documented. The induction of mutations at specific sites in some proto-oncogenes and tumour suppressor genes, resulting in transformation of normal cells into tumorous ones, provides a mechanistic basis for this strong association. People with inherited conditions or who have been subjected to environmental exposures that make their DNA unstable, manifested as chromosomal aberrations, also have a high risk for certain cancers.

Identification of an agent as a mutagen in one or more of the currently used tests for genetic toxicity should trigger a series of actions, including:

- an assessment of the probable exposures of humans and other biota
- an assessment of the agent's environmental stability and distribution
- an attempt to determine the potency of the agent to a wide range of organisms

This information can then be used to manage any environmental risk the agent presents. For most mutagens, risk management programmes include:

- restricting environmental release
- taking preventive measures against exposure
- proper labelling

Even with such programmes, organisms with unique hypersensitivity to certain mutagens may still be at risk. The minimal data base required for risk management programmes should be identified.

GENETIC RISK ASSESSMENT

For agents to which there is substantial human exposure but which must be tolerated in the environment, some type of genetic risk assessment is desirable. The methods reviewed in this document are applicable for this purpose. The risk–benefit ratios obtained, including formal risk assessments, are valuable in determining the probable effects of the agent on health and are necessary for proper communication of the risks. As the resources required to conduct a genetic risk assessment are considerable, however, such assessments can be done in only a few circumstances.

One of the few agents that has been studied extensively for genetic risk in both experimental animals and humans is ionizing radiation. Data from those studies are useful for learning how to extrapolate from animal models to the human situation. Results from studies of genetic risk of chemicals cannot, however, be compared directly to results from studies of radiation because of unique factors in the molecular dosimetry of chemicals:

- exposure and dose are not related in a constant way
- chemicals undergo metabolism, distribution and excretion

The relationship between the risks due to radiation and those due to chemicals is an area for fruitful future study.

The proper management of genetic risks associated with exposure to agents that induce mutations in genes or chromosomes should thus be based on what is known about the ability of the agent to reach the DNA of somatic and germ cells and the benefits and associated costs required to control exposure of the target species to the agent.

Recommendations

The following recommendations are intended to emphasize specific concepts and advice given in the preceding parts of the document, and to identify areas in the identification of genetic hazards and assessments of their risks which must be developed further before this science can be applied more effectively to humans and other populations of living organisms.

INFORMATION AND TECHNOLOGY

More extensive knowledge about the quantitative and qualitative relationships between mutations induced in somatic cells and germ cells of humans and other mammals

This information would permit prediction of the risk to germ cells on the basis of the results of tests using somatic cells, which are much faster and cheaper than current methods available for analysing germ cells.

Better understanding of the role of genetic damage in the aetiology of somatic and heritable diseases in humans and other biota

This information is needed to support the assumption that environmental mutagens represent significant risks to health.

Improved methods for surveillance of genetic damage in human populations

Such techniques will permit reliable assessment of human populations subjected to accidental or other severe environmental exposures for evidence of adverse genetic effects.

Improved animal models for assessing risk to somatic and germ cells

The animal model systems used currently in risk assessment entail considerable resources and cannot therefore be used widely. New models, such as transgenic mice in which gene mutation can be detected in all tissues, including germinal tissue, appear to be relatively cost-effective and adaptable.

GENETIC RISK ASSESSMENT

Better means of expressing genetic risk in animal models as estimates of increased disease burden in humans

Currently used models for risk assessment do not allow translation of data on mutations in animal germ cells into quantitative estimates of increase in disease frequency in exposed human populations. This deficiency must be resolved if quantitative risk estimation of genetic effects is to become applicable to the regulation of environmental agents.

Closer consideration of local priorities in risk assessment and management reponses to identification of a genetic hazard in non-human biota

Quantitative risk assessments are not relevant for non-human biota, owing to the tremendous diversity of these organisms and the absence of information about the relevance of somatic-cell mutations in most of them.

Identification of a genetic hazard should trigger a risk management response based on the locally available resources and the economic impact of leaving the agent in the environment.

Development of decision criteria before initiation of quantitative risk assessment of heritable mutations in human populations

Because of the absence of epidemiological data to support the concept that environmental mutagens are responsible for human diseases due to germinal mutations, it is recommended that decision criteria be developed before the substantial resources required to assess genetic risk are deployed. The criteria might include the specific circumstances required and considerations of the costs and benefits associated with the decision.

GLOBAL ISSUES RELATED TO GENETIC RISK

Geographic mapping of the prevalence of genetic diseases in humans

Registration and geographic mapping of the incidence of human diseases caused by genetic damage may provide valuable information about the aetiology of these diseases and about temporal shifts in disease incidence, which are relevant to the deployment of health care resources.

Monitoring to evaluate the transport and deposition of genotoxins

Biomonitoring of a range of non-human biota and monitoring of the transport and deposition of genotoxins in air and water may provide insight into changes in levels of risk observed in specific geographical locations. Regional monitoring sites might be established to provide actual data.

Centralized preparation and distribution of monographs on genetic hazard and risk

The resources for critically assessing and evaluating possible genetic hazards and risks are limited. It is recommended that monographs in which specific chemicals or classes of chemicals are assessed be produced and distributed from a central source which has the necessary expertise and resources. This would reduce the need for duplication of limited resources, while providing high-quality, authoritative evaluations.

Many of these recommendations could be implemented rapidly and begin to yield valuable data. Development of the technical and information requirements could be sponsored by national agencies that fund health and environmental activities. Global issues and considerations of implementation will require support that is based more broadly. These might be areas in which international agencies and programmes, such as the International Agency for Research on Cancer (IARC), the International Programme on Chemical Safety (IPCS), the United Nations Environment Programme (UNEP) and the International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC), might usefully become involved.

Assessing the Risk of Genetic Damage

Hodder & Stoughton

ISBN 92-807-1339-6

Imprimerie MONTBLIS, Saint-Léonard