Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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3 *Methods for Quantitative Estimation of Risk from Exposure to Chemicals**

3.1 INTRODUCTION

There are two main sources of information on adverse effects of exposure to chemicals: laboratory experiments on whole animals or other test systems, and human studies which include epidemiological surveys and clinical investigations. Generally, laboratory animals are exposed to much higher levels of chemicals than are human individuals and populations. Estimation of response at relatively low-level environmental exposures necessarily involves the extrapolation of data obtained at high doses. When toxicity information is derived from studies on experimental animals, biological extrapolation or transposition is required to estimate human response. The reliability of biological extrapolations depends on the knowledge of comparative biology and toxicology which is at present very limited.

In the context of the present report, risk means the expected frequency or probability of an effect that is undesirable or harmful to health. Much of the available information on risks from exposure to chemicals has been obtained in cancer research, and therefore, the main emphasis in this report is on methods for estimating cancer risk.

3.2 CARCINOGENESIS AND MUTAGENESIS

Cancer risk assessment is a two component process involving a qualitative judgment of how likely it is that an agent is a human carcinogen, and a quantitative judgment on how much human cancer the agent is likely to cause at given levels and durations of exposure.

Risk assessment may be based on animal or human evidence of cancer incidence associated with exposure to chemicals and requires a judgment of the evidence of carcinogenicity based on the scope and quality of the available studies and the nature of response. There are various approaches to stratifying

* This part of the Joint Report was prepared under the chairmanship of David G. Hoel by 4 working groups listed in footnotes to Sections 3.2.1, 3.2.2, and 3.2.3.

the weight of evidence for carcinogenicity of chemicals but only the one adopted by the International Agency for Research on Cancer (IARC, 1982) has international acceptance. Results of short-term tests, such as those for mutagenicity, are generally regarded as providing support in the assessment of cancer risk.

Although human data are the most relevant for cancer risk assessment, negative epidemiological evidence of carcinogenicity in the face of positive animal evidence is not necessarily an indication of the lack of human responsiveness. It may simply be due to the insensitivity of a particular epidemiological study. Negative epidemiological results, when combined with exposure estimates, can be used to define statistical upper limits for cancer risk; such information can be a valuable adjunct to risk estimates. Generally, greater weight is given to positive response in animals than to negative results because of the wide variability in animal sensitivity.

Quantitative risk estimation generally includes levels of exposure far below those which can be observed in animal studies or most epidemiological investigations, and therefore it requires an appropriate mathematical formulation of the relationship between dose and response on which low dose extrapolation can be based. There are many mathematical models which fit tumour response data in the observed range but give widely divergent estimates at low doses. The justification for any mathematical model ultimately rests on the description of the biological processes underlying carcinogenesis which the model provides.

Estimates of cancer risk based on animal studies do not usually take into account the possibility of synergistic interactions with other environmental factors. Although several examples exist of synergistic interaction in humans, such as cigarette smoking and asbestos, the importance of synergism at low levels of exposure to carcinogens is not known. Estimations of cancer risk based on human data may encompass interactions with other substances in the environment of the type which may or may not be relevant to the population for which the risk estimate is made.

Cancer risk can be expressed as risk in an individual, i.e. the lifetime excess probability of incurring cancer as a result of an exposure to environmental agents. It can also be expressed in terms of risk in the exposed population, i.e., the number of additional cancer cases per year caused by a given level of carcinogen. Carcinogen exposure in a population can be controlled so that the number of excess cancer cases may be negligible; however, for a few individuals in the exposed population the risk may still be unacceptably high. Conversely, in very large exposed populations, the individual risk can be acceptably low but the excess number of cancer cases may be unacceptably high. It is obviously desirable to control carcinogen exposure to minimize both risk in individuals and in populations.

3.2.1 Cellular and Molecular Basis of Quantitative Risk Estimation*

Undesirable or harmful biological effects resulting from exposure to chemicals, such as mutations and cancer, and the application of this knowledge to risk estimation must be considered both at the level of the whole organism and at the level of various organs, tissues, cells and molecules, with particular reference to structural alterations of DNA and other informational macromolecules. In order to develop more adequate methods for quantitative estimation of risk in humans based on data obtained in laboratory animals and other experimental systems, a detailed understanding is required of the mechanisms of biological action of various chemicals. Even if this goal may be scientifically achievable, there is no assurance, at the present state of knowledge, that reliable quantitative predictions can eventually be made.

3.2.1.1 Genetic Effects

Genes in both germ cells and somatic cells are responsible for maintaining genetic characteristics of a species and the phenotype and various functions of an organism. Heritable alterations in the nucleotide sequences of genes which code for regulatory or structural proteins are considered as mutagenic events. Mutagenesis is a process which leads to various types of gene or chromosome mutations, and chemicals which contribute to mutagenesis are considered as mutagens. Changes such as unrepaired DNA damage in non-replicating cells could alter the fidelity of transcription of a particular DNA sequence or affect the expression of another non-damaged gene. In this case, DNA lesions induced by chemicals could lead to harmful phenotypic changes in non-replicating somatic cells. However, from the classical genetics viewpoint they would not be considered as 'genetic effects' because they are not heritable since mutations in somatic cells, in contrast to mutations in germ cells, are not transmitted from generation to generation.

Types of Mutations Changes in the quality and quantity of DNA can be of several types. Base changes, deletions, additions, translocations and transpositions of DNA sequences, as well as changes in the chromosome structure and partial or whole-set changes in chromosome number, can all be considered as mutations.

Mechanisms leading to these qualitatively different types of gene and chromosome mutations are poorly understood in any biological system. However, it seems reasonable to assume that there are different mechanisms leading to various classes of gene and chromosome mutations. Some chemicals

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which can produce one or more types of base damage or strand breaks, could cause one or more types of gene or chromosome mutations. In addition, mutations, at the gene and chromosome level, can be induced by chemicals which do not damage DNA directly, but produce any one of the following effects: reduce the fidelity of replication of normal DNA by altering nucleotide pools or affecting enzymes involved in DNA replication; inhibit the error-free repair of DNA damaged by other agents; affect the ability of cells to repair DNA, for example, by triggering cell replication with unrepaired lesions in the DNA template; or lead to chromosomal replication (endoreduplication) or segregation (aneuploidy).

The majority of non-repaired, persistent modifications introduced into DNA by carcinogens are localized in transcriptionally silent parts of the genome. They become effective only when the functional integrity of the respective DNA sequences is 'put to the test' in the course of gene activation or inactivation. For example, this could occur by further progression of cells along a pathway of development and differentiation, by inducing cells to express specialized functions, or after cells enter the cell cycle from a non-proliferative state (Hecker *et al.*, 1982).

Besides local alterations of nucleotide sequence, structural modifications of chromosomal DNA may lead to helical distortions, and in certain cases facilitate the transition of the B-form of the double helix to a left-handed conformation (Z-DNA) (Wang *et al.*, 1979; Santella *et al.*, 1981). Persistent chemically modified DNA components could also interfere with the patterns of mRNA processing (gene splicing) or DNA methylation, affect the precision of DNA rearrangements possibly associated with development and differentiation in mammalian cell systems, cause inappropriate gene amplification and rearrangements at the chromosome level, and perhaps induce error-prone DNA repair (Radman *et al.*, 1977; Grunberger and Weinstein, 1979; Rajewsky, 1980; Lavi, 1981; Pfohl-Leszkowicz *et al.*, 1981). The common denominator appears to be the interference with the particular genetic programmes of the respective target cells, resulting in an inappropriate expression, or degree of expression, of cellular *onc* genes (Hayward *et al.*, 1981; Lane *et al.*, 1981; Weinberg, 1981, 1982; Cooper, 1982; Reddy *et al.*, 1982; Tabin *et al.*, 1982).

Measurement of Mutations Mutations are ultimately measured by determining if DNA sequences have been altered qualitatively or quantitatively, if proteins coded by certain DNA sequences have been modified or if the number or morphology of chromosomes have been changed. Indirectly, the influence of some mutated genes can be detected by changes in cellular phenotypes. However, this way of measuring mutations presupposes that all genes which have been mutated will bring about a detectable phenotypic change, and that all detectable phenotypic changes are due to mutations. Clearly, both theoretical and experimental evidence indicate that some mutagenic events will not be detected

by available phenotypic markers, and that some stable phenotypic changes might not necessarily be the result of gene or chromosome mutations. Also, a mutation in a regulatory sequence of DNA (or an unrepaired DNA lesion in a non-replicating cell or in a transcriptionally silent region of a replicating cell's genome) might indirectly alter the expression of a normal gene.

Techniques have been devised to analyse:

(1) lesions which could act as substrates for mutations (for example, antibodies to specific chemically induced DNA lesions),
(2) the processes by which the repair, or lack thereof, could produce altered DNA (DNA damage or DNA repair assays, *in vitro* or *in vivo*),
(3) processes by which repair, replicating enzymes or nucleotide precursors could influence mutagenesis ('fidelity' assays),
(4) changes in DNA sequence (characterization of restriction endonucleases of known DNA sequences),
(5) changes in gene products (two-dimensional, computer-assisted chromatographic analysis of polypeptides and amino acid sequences), and
(6) phenotypic markers for mutations, *in vitro* and potential *in vivo*.

Until recently, specific reaction products of chemicals with DNA could only be identified by radiochromatography of hydrolysed DNA after the application of radioactively labelled indicators (Baird, 1979). High affinity and specific monoclonal antibodies have now become available for sensitive detection and quantification of structurally modified DNA components even at the level of individual cells (see Müller and Rajewsky, 1981; and Rajewsky, this volume). In addition to recently developed 'post-labelling' techniques (Randerath *et al.*, 1981), these new methods will considerably facilitate the analysis of DNA in small samples of cells after exposure to non-radioactive chemicals.

3.2.1.2 The Process of Carcinogenesis

Multistage Nature of Carcinogenesis There is considerable evidence that the process of malignant transformation (with subsequent tumorigenesis) occurs through several qualitatively different stages. Support for a multistage model of carcinogenesis is provided by many diverse studies including the pathological examination of tumours, experimental carcinogenesis, dominant inheritance of certain tumours, epidemiological studies and experiments with cells in culture (Foulds, 1954; Laerum and Rajewsky, 1975; Barrett and Ts'o, 1978). The exact number of stages involved in the process of carcinogenesis is unknown, although at least two stages have been identified (Berenblum, 1975). Of course, a different number of stages may be needed for different types of target cells, or even for malignant transformation of the same cells following treatment with different inducing agents (see Barrett and Thomassen, this volume).

Various multihit and multistage models have been proposed, primarily based on epidemiological data on human tumours, which indicate a strong time dependence of tumour incidence (see Armitage and Doll, 1954; Druckrey, 1967; Cook *et al.*, 1969; Moolgavkar and Knudson, 1981; Hoel, this volume). A simple multihit model implies that a number of heritable changes (up to 7) must be accumulated in a cell prior to its malignant conversion. The tumorigenic cell then proliferates clonally to form a tumour. It is generally assumed that these changes occur independently.

A multistage model (Figure 3.1) differs from a multihit model in that the probability of subsequent stages in the development of malignant state is increased by the clonal proliferation of altered but not yet fully malignant cells (Trosko and Chang, 1980; Potter, 1981; Moolgavkar and Knudson, 1981). Since the number of altered cells may thus increase exponentially with time, such models can describe epidemiological data with as few as two steps whereby competing factors of division and differentiation of the intermediate cells may also affect clonal expansion.

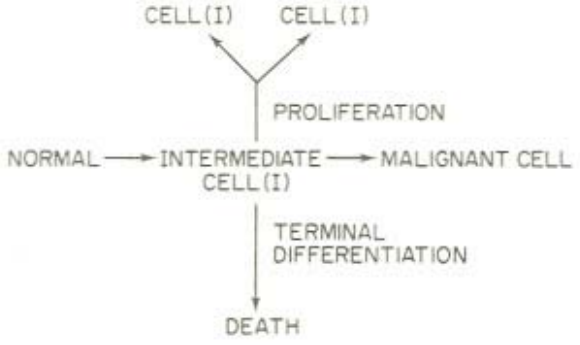

Figure 3.1 Multistage model of carcinogenesis

Multistage models are compatible with the influence of multiple factors in carcinogenesis. The transition of a normal cell to an intermediate stage is the initial event in carcinogenesis and can be caused by a variety of chemicals. Secondly, the clonal expansion of intermediate cells can be influenced by agents which either stimulate the division of cells, or inhibit their differentiation, or both. Thirdly, the transition of the intermediate cells to the neoplastic or malignant state could be influenced either by the population size, or by substances which increase the rate of transition of these cells to a tumorigenic state (Trosko and Chang, 1980; Potter, 1981; Moolgavkar and Knudson, 1981; Barrett and Thomassen, this volume). While this is of course the simplest multistep model that can be proposed, the addition of further steps in the process, or of agents which influence the clonal expansion of the malignant cells,

would only reflect a larger number of factors that may play a role in carcinogenesis. The factors may be unique for each stage in the process. Hence, the progressive multistage nature of malignant development provides a basis for the understanding of some of the multiple risk factors in carcinogenesis.

Initiation and Promotion The concepts of initiation and promotion were originally formulated by Berenblum (1941), Rous and Kidd (1941), Mottram (1944) and Berenblum and Shubik (1947) to explain the multistage nature of mouse skin tumorigenesis. The fundamental aspects of multistage carcinogenesis are illustrated schematically in Figure 3.2, and the basic properties of tumour initiators and promoters are listed in Table 3.1. It is evident that the action of initiators is irreversible, whereas some of the biological effects of promoters can be reversible. In terms of mechanism, it is of interest that initiators, or their metabolites, generally bind covalently to DNA and other cellular macromolecules and are, therefore, mutagenic (see Grover, 1979; Hollstein *et al.*, 1979; Weinstein *et al.*, 1979b; Trosko and Chang, 1981; Yamasaki and Weinstein, this volume).

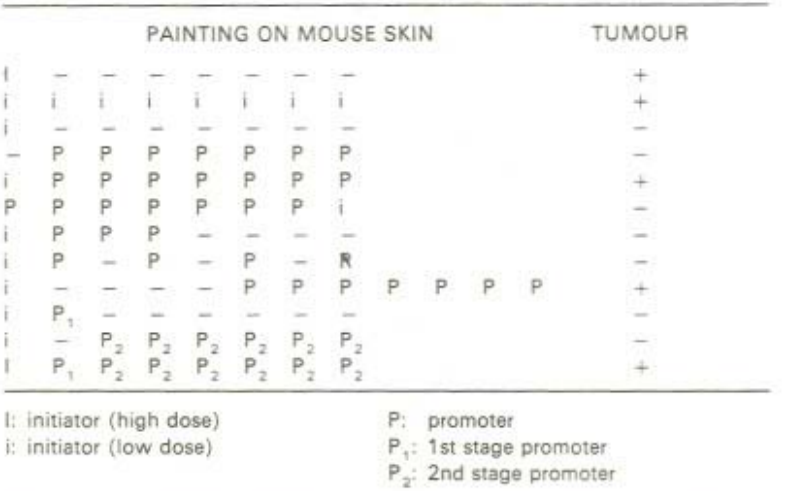

Figure 3.2 Schematic diagram of multistage mouse skin carcinogenesis

Although there is no evidence for the covalent binding of tumour promoters to DNA, promoters have been shown recently to induce DNA strand breaks in human lymphocytes *in vitro* (Birnboim, 1981), sister-chromatid exchanges in cultured mammalian cells (Kinsella and Radman, 1978; Nagasawa *et al.*, 1983) and mitotic aneuploidy in yeast (Parry *et al.*, 1981), thus adding confusion to the understanding of the mechanism of tumour promotion. Other studies suggest

Table 3.1 A comparison of biological properties of initiating and promoting agents

Initiating agents	Promoting agents
(1) Carcinogenic by themselves	(1) Not carcinogenic alone
(2) Must be given before promoting agents	(2) Must be given after the initiating agent
(3) Single exposure is sufficient	(3) Require prolonged exposure
(4) Action is irreversible and additive	(4) Action is reversible (at early stages) and not additive
(5) No apparent threshold	(5) Probable threshold*
(6) Yield electrophiles that bind covalently to macromolecules	(6) No evidence of covalent binding
(7) Mutagenic	(7) Not mutagenic

* May depend on the time intervals of promoter applications

that the primary action of tumour promoters takes place at the cellular membrane (see Weinstein *et al.*, 1979a; Blumberg, 1980, 1981; Hecker *et al.*, 1982). Such differences may explain why the promotion stage is reversible, whereas the initiation is not.

Recent experimental studies on rat and mouse liver, rat bladder, rat mammary gland and rat colon as target tissues appear to be consistent with the two-stage model (see Hecker *et al.*, 1982; Yamasaki and Weinstein, this volume). In particular, there is an accumulating evidence that liver carcinogenesis in rats occurs by a multistep process (Pitot and Sirica, 1980) and so does the carcinogenesis on mouse skin (see Boutwell, 1974; Yamasaki and Weinstein, this volume).

The agents which act as tumour promoters in two-stage carcinogenesis are chemically diverse, and include such compounds as phorbol esters, saccharin, phenobarbital, tetrachlorodibenzo-*p*-dioxin (TCDD) and DDT. However, much of our recent knowledge about the mechanism of action of tumour promoters has come from the study of phorbol esters and their derivatives, using either cell cultures or mouse skin (Hecker *et al.*, 1982; Yamasaki and Weinstein, this volume). Thus, our understanding of the mechanism of tumour promotion is essentially limited to this class of compounds. The process of tumour promotion involves a proliferation of initiated cells as compared to non-initiated cells. The observation that tumour promoters are often hyperplasiogenic could explain the clonal expansion of initiated cells. However, unless there is a retardation in the maturation of initiated cells compared with the surrounding normal cells, one would not see the accumulation of a clone of initiated cells. Several studies have shown that phorbol esters affect gene expression and cell differentiation in a variety of cell types *in vivo* and *in vitro* (Diamond *et al.*, 1978; Yuspa *et al.*, 1982).

It has been proposed that aberrant cell differentiation is involved in the mechanism of tumour promotion. Several biochemical mechanisms of tumour promotion by phorbol esters have been proposed (Murray and Fitzgerald, 1979; Yotti *et al.*, 1979; Weinstein *et al.*, 1980; Troll *et al.*, 1982; Yamasaki and Weinstein, this volume).

The process of tumour promotion in mouse epidermal carcinogenesis can itself be subdivided into different stages. Potent tumour-promoting substances, such as croton oil or 12-*O*-tetradecanoylphorbol-13-acetate (TPA), can effect changes in initiated cells which allow them to be multiplied by compounds which are inactive or weak promoters by themselves ('second stage promoters') (Slaga *et al.*, 1980a). Different inhibitors of promotion can be shown to be effective in hindering only the first or second stage of promotion (see Slaga *et al.*, 1980b). These results suggest that potent promoters can modulate or convert initiated cells in a qualitative manner to allow them to proliferate into a clone of tumour cells in response to a second-stage promoter. Thus, the properties of first-stage promoters are different from the properties of complete promoters; for example, first-stage promoters need to be applied only once and their effects are persistent, at least for a defined time period. The two-stage model of mouse epidermal carcinogenesis only describes the development of benign tumours. To describe the complete process, a further progression of cells to the malignant stage has to occur. The nature of the driving force in malignant transformation is undefined although it is known in mouse skin carcinogenesis that the development of carcinomas is independent of the continued exposure of the cells to promoters (see Boutwell, 1974; Yuspa *et al.*, 1982). The influence of other mutagenic chemicals remains to be determined.

Probability of Mutagenesis and Malignant Transformation at the Cellular and Molecular Levels A large proportion of chemicals require activation, i.e. conversion by cellular enzymes to their ultimate reactive derivatives (Miller and Miller, 1979). The ability for enzymatic activation varies between individuals, tissues and different types of cells. Even within a given cell lineage, enzyme activity may vary as a function of the stage of development/differentiation. The cellular capacity to activate chemicals to their ultimate reactive derivatives, therefore, constitutes the first determinant of the probability of mutagenesis or carcinogenesis.

The reactive forms of most chemical carcinogens are electrophilic, they react with nucleophilic sites in DNA and are usually mutagenic. This does not, however, constitute a proof that mutation is an obligatory requirement for malignant transformation. Cellular macromolecules other than DNA also contain multiple nucleophilic sites and react with electrophiles. None the less, the central importance of DNA for the expression of genetic information provides a strong argument for a critical role of DNA modification in the process of chemical carcinogenesis.

Some mutagen/carcinogen–DNA adducts can be specifically recognized, removed, and repaired by cellular enzymes. The capacity of mammalian cells to perform these processes varies considerably not only between species and individuals but also between different tissues and cells of a given organism. This differential capacity of cells to repair DNA lesions is the second determinant of the probability of mutation and malignant conversion.

The 'fixation' of mutations caused by chemical modifications of DNA requires the persistence of these modified structures until the completion of DNA replication, i.e. it only occurs in cycling cells or in resting cells that have retained the capacity for proliferation (Trosko and Chang, 1981; Rajewsky, this volume). Likewise, there is no evidence that cells can undergo malignant transformation by exposure to chemical carcinogens after having irreversibly reached a terminally differentiated, non-proliferative stage. Therefore, the third determinant of the probability of mutation or malignant conversion is the proliferative state of the target cell at the time of exposure.

Furthermore, there is evidence to suggest that specific precursor cell stages in the pathway of development and differentiation of a given cell exist where the gene programme can be shifted to the expression of malignant phenotypes with higher probability than would be expected for a random process (Rajewsky *et al.*, 1977; Graf and Berg, 1978; Graf and Jaenisch, 1982). A cell type- and development/differentiation stage dependence of the relative transformation likelihood may, therefore, constitute the fourth determinant of probability of malignant conversion, possibly varying with the type of chemical carcinogen applied.

As carcinogenesis is a multistage process, the probability that cells will progress through post-initiation stages of the process may, therefore, be differentially influenced by a fifth determinant, namely their respective microenvironment which, in turn, can vary as a function of the host age, immune status, hormonal and other physiological control factors, intercellular contacts and communication.

3.2.1.3 Conclusions

(1) There are many biological and biochemical processes leading to mutations, and, therefore, no single technique can be expected to measure all cellular changes related to mutagenesis.

(2) Carcinogenesis is a multistage process that can be influenced by a variety of factors. A given agent may affect more than one stage in this process, and different factors may act synergistically. An agent may inhibit or promote specific stages in carcinogenesis, and the same agent may act differently under different conditions.

(3) The presumed mutational event in initiation may be related to alteration of DNA, possibly in the sense of a gene mutation or a chromosome mutation.

Chromosome mutations may not be detected by standard assays used to detect mutagenic agents.

(4) Tumour promoters are generally not mutagenic. However, mutagenic substances can be tumour promoters through mechanisms such as cell killing.

(5) There is evidence in certain cases that human carcinogenesis may also be a multistage process. Since the human organism is exposed to numerous environmental agents, multifactorial carcinogenesis, in which two or more agents act synergistically, may be an important consideration in the assessment of cancer risk in humans.

(6) The probability of malignant transformation depends on several determinants at the level of individuals, organs, tissues, and cells. Among these are:

(a) the capacity of cells to enzymatically convert environmental chemicals into their reactive, generally electrophilic derivatives;
(b) the capacity of cells for enzymatic removal and repair of chemically modified DNA components which, if persistent, might have mutational and other genetic effects;
(c) the proliferative and development/differentiation state of the target cells; and
(d) the micro environment of the presumptive tumorigenic cells.

3.2.1.4 Recommendations

(1) As there are non-mutagenic factors influencing carcinogenesis, tests must be designed to detect non-mutagenic chemicals which may be harmful to human health.

(2) The initial event in the process of carcinogenesis is often induced by mutagenic agents. However, there is no absolute proof that the mutation itself causes initiation. Although many authors favour mutational mechanism, 'epigenetic' alteration in gene expression may also be a critical event in initiation. Further studies are needed to clarify this issue.

(3) The promotion phase of carcinogenesis involves the clonal expansion of initiated cells. This can be influenced by non-specific tissue injuries (for example, wounding, hyperplasia) and by specific chemical compounds. Potent chemical tumour promoters affect gene expression, cell-to-cell communication and cellular differentiation, which may be important factors in the efficacy of these substances. This aspect requires further research.

(4) The later stages of carcinogenesis can be influenced by promoting agents, and possibly by mutagenic agents if these increase the rate of transition of cells to a malignant state. It is possible that chemicals exist that are neither initiators nor promoters, but only affect or alter some transition stage in the process of malignant progression. Classification of a chemical as an 'initiator' which accentuates the early effects of the chemical, often overshadows promotional

influences of the agent in later stages of the carcinogenic process. Further studies are required to improve our knowledge of the role of exogenous chemicals on later stages of carcinogenesis.

(5) Mouse two-stage carcinogenesis and other models indicate that tumour promotion is, to some extent, reversible. It has, therefore, been considered that there exists a threshold or no-effect level for tumour promoters. However, the reversible nature of tumour promoters only gives a theoretical basis for a possible threshold, but there is no conclusive experimental evidence to show the existence of a threshold dose in tumour promotion. More research is needed in this area.

(6) To understand fully the process of carcinogenesis, more information is required on the mechanism and control of gene expression and cell differentiation in normal cell systems, and on the mechanisms responsible for the stringent maintenance of ordered tissue structure.

3.2.2 Laboratory Models for Carcinogenesis and Mutagenesis*

Animal experimental data can be used for risk estimation in humans only if the assay for carcinogenicity has been adequately designed and executed so that it yields reliable qualitative and quantitative information. As basic requirements for long-term and short-term assays for carcinogenicity have been reviewed recently (IARC, 1980), only some issues are discussed in this section.

A proper definition of the test substance is essential. Zbinden (1973) specified 14 chemical and physical properties required to define a new drug. An important consideration is the content of impurities that may be present either as byproducts of manufacturing procedures or as a result of decomposition of an unstable test substance.

For studies of toxicity mechanisms the choice of experimental organisms and biological materials is large: micro-organisms, yeasts, invertebrates, lower vertebrates, and higher species, including cell and organ cultures. For risk-related studies there is advantage in selecting organisms which are phylogenetically nearer to the human species, mammals being the most widely used.

Rodents are often the animals of choice because of their well-defined characteristics, uniformity of genetic background, easiness to breed, reasonably short life-span and low cost. The use of two or more related or unrelated species is often an advantage. The selection of the appropriate strain may also pose a problem when several well-characterized strains are available. In general, it is convenient to select those strains where the background incidence of tumours is low, so that small increments in incidence can be detected; this emphasizes the importance of previous experience with a given strain. Inbred animals may be considered to have advantages because of genetic homogeneity which reduces the variability of observations. From the viewpoint of comparability with the human

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species, which is genetically highly heterogeneous, the use of single strains of inbred animals may be a serious limitation. These considerations apply both to *in vitro* assays and to short-term assays or metabolic studies on animal tissues and cells. The development of human cell and tissue culture methods makes it possible to undertake comparative mechanism studies on both human and animal targets.

Many chemicals are often not carcinogenic or mutagenic *per se* but require metabolic conversion to reactive intermediates. Animal species that metabolize the test substance in a similar way to man provide an obvious advantage although this may be difficult to establish because the information on comparative biochemistry of different animal species is limited.

Comparisons of toxicity of chemicals is simplified if the underlying mechanisms of action are similar. It is then more likely that the kinetics of toxic action and of repair or progression of the induced lesion are similar as well. Although the exact mechanism of carcinogenic action is not known, there is evidence that carcinogenesis is a multistage process which requires a considerable time for expression. There is also evidence that somatic mutations may contribute to at least one of the stages. Mutation is the basis of many short-term tests; there are, however, carcinogenic chemicals (for example, hormones) which are not mutagenic ('epigenetic' carcinogens) and others whose effects on the genome may not be detected by some mutagenicity tests.

3.2.2.1 Long-term Whole-animal Assays as Predictors of Chemical Carcinogenesis in Man

Long-term assays are considered here as a source of quantitative data on carcinogenicity of chemicals needed for estimating cancer risk in humans. The purpose of assays influences the selection of biological models, test doses and experimental conditions, and the choice of treatment and observation protocols.

The sex of the animals is often not a critical consideration, except when hormonal effects are investigated or when sex-related tumours have such a high incidence as to seriously affect the outcome of the test, for example, mammary and testicular tumours. Male and female groups are evaluated separately and the results may be combined only in the absence of any demonstrable differences between sexes.

The age of the animals is important in view of the age-dependent differentiation of some target cells or tissues.

Ideally, the number of animals assigned to a group should be greater for low than for high dose groups, so that the number of 'positive effects' scored is kept approximately equal within the whole range of doses studied. However, particularly at low doses, the statistical significance of differences from the controls may be difficult to demonstrate, although the significance of the trend over a range of doses may be established.

It is important to point out that most of the major forms of human cancer have been obtained in animal models by exposure to chemicals, and that there is a close qualitative similarity between human cancer pathology and the corresponding pathology in animal models. There are, however, some animal tumours resulting from various chemical treatments that are quite different from any human tumour counterpart, for example, lung adenomas in mice and Zymbal gland tumours in rats. These tumours can be useful indicators of the overall carcinogenicity of a test compound, but are not appropriate endpoints if one is interested in tumours that could result from chemical exposure of human organism.

Purpose of Tests and Selection of Models Animal carcinogenesis tests designed for the general purpose of identifying possible carcinogenicity of hitherto untested compounds often do not include more than two dose levels of the test compound (NCI, 1976), and do not provide as good an indication of the dose–response relationship as can be obtained with a protocol using more dose levels. When such screening tests include a dose level that represents the estimated maximum tolerated dose (EMTD) or a dose close to it, they can at least provide an upper limit of detectable response under the selected test conditions. If there are other competing risks, however, the maximum level of carcinogenic response may not occur at the EMTD. This is particularly noteworthy when different doses result in distinctive tumour types, especially if they vary in their latent period (time-to-occurrence) and lethality. Lower dose groups surviving longer without tumours with a short latent period may develop a significant number of tumours arising late in the life-span of the species (Maltoni, this volume). Thus, the selection of dose levels in whole-animal assays is still a controversial issue (Food Safety Council, 1978), the main point of contention being the use and estimation of the EMTD (IARC, 1980). There is an urgent need for a test design which would be equally efficient in detecting a carcinogen as is the current assay with two doses, but which would provide better information on dose–response relationship without significantly increasing the total number of test animals.

Some carcinogenesis models are particularly suitable for the study of factors that relate to the pathogenesis of special tumour types, for example, models for the induction of bronchogenic carcinomas by carcinogens transported by carrier particles (Saffiotti *et al.*, 1968), and models for the induction of neurogenic tumours by treatment of rats during prenatal development (Rajewsky, this volume). Special bioassays can also be designed to obtain a detailed dose–response relationship for certain tumour types by using species, strains, routes of administration and test conditions that are particularly appropriate for the induction of a specific type of tumour (for example, SENCAR mice for skin tumours, and strain A mice for lung adenoma).

It is generally recommended that exposure to the test substance should be initiated a few weeks after the weaning and then continued for the major part of

the animal's life. The usual duration of experiments for rats is 24 months and for mice at least 18 months. Some authors consider that the test period should cover the entire life-span of the animal (Maltoni, this volume), but the issue has not been resolved.

The detectability of the effect at any dose, particularly at low doses, is obviously related to the incidence of tumours at zero dose level. In general, the observation of tumours having a high background incidence and a late time of appearance (for example, the reticulum cell sarcoma in some strains of mouse) is more difficult to interpret than the observation of low-incidence tumours having a short latency time (for example, thymic lymphoma in some other strains).

The tumour incidence rate and the total tumour incidence are the most common endpoints when dealing with single tumours, but tumour multiplicity is a phenomenon frequently observed. When this is the case, the number of tumours per animal as well as the percentage of tumour-bearing animals should be plotted against the dose to describe the kinetics of tumour development. At very high doses the first type of plot does not show saturation, while the second does. However, at low doses of interest for risk estimation the shapes of the two curves are essentially the same.

Long-term studies should not be carried out without careful macro- and microscopic pathological examinations. Although a good deal of subjective judgement is involved in identifying different tumour histotypes and in establishing causes of death, pathological examination is essential for evaluating any study.

Although various types of tumours may sometimes be grouped together for comprehensive evaluation, it must be emphasized that each tumour type may have a specific pathogenic mechanism, that there is species specificity for tumour induction and that pathological entities classified under the same heading in animal and man may have widely different nosographic characteristics.

In analysing tumour induction experiments, attention should be paid to the tumour spectrum which characterizes each species and strain. This knowledge may help in selecting, at the design stage, the most appropriate strain for testing and may provide guidance for the interpretation of results in the final stage of the analysis.

When accounting for tumour frequencies, it is not a good practice to group together different types of tumours because the induction of some tumours could be masked by a relative decrease of other types of neoplasms.

The selection of protocols for quantitative studies is influenced both by qualitative and quantitative criteria, since precise quantitation of the response in a system that is qualitatively inadequate may be irrelevant or even misleading. Qualitative criteria for the selection of an appropriate test protocol for quantitative studies include not only species, strain and sex, but also the mode of administration and tissue specificity of the test substance, vehicle, dietary regimen and some other variables. Quantitative aspects are also critical in the

selection of optimal test protocols, including randomization of test animals among treated and control groups and the selection of optimal group sizes and test doses.

The size of groups at the beginning of the treatment and, what is possibly even more important, the size at the time of expected tumour response, is critical in determining whether the number of tumours can be estimated with sufficient precision. The size of groups needs to be determined for each test both as regards the number of animals exposed and the number of controls.

The selection of a model for quantitative study of carcinogenesis intended for use in estimating risk in man may be considerably influenced by the degree of comparability of the animal and human target tissues and of their pattern of response to carcinogens as determined by morphological and functional studies. Quantitative studies for use in risk estimation may include histogenesis and histopathology, identification of cells of origin of tumours in a given tissue, histochemical and immunochemical markers, ultrastructure, cytokinetics, examination of the early cellular response to carcinogens in tissue explants from human subjects and the corresponding animal models, metabolic investigations of the capability of target organs and cells to activate and bind the test substance or related substances, and the identification of specific molecular lesions, such as carcinogen–DNA adducts.

The development of methods for the study of human tissues in culture and their response to carcinogens has widely extended the basis for qualitative and quantitative comparison of response to carcinogens in human subjects and in animal models (for reviews see Harris *et al.*, 1980, 1982).

Variability Factors The general methodology of long-term assays is reasonably well established and in recent years it has become more standardized. There remain, however, numerous variables which may influence the quantitative outcome of such bioassays. Some can be avoided by good laboratory practices.

Infections and parasitic infestations can alter the level of response to carcinogens by various mechanisms (for example, stimulation of cell proliferation in target tissues, interference with the survival rate of test animals, masking of a pathological effect). The wider use of pathogen-controlled environments in the breeding and housing of experimental animals has greatly reduced the confounding effects of such variables.

Some variables are of particular relevance when comparing levels of response in tests conducted in different laboratories, or even in the same laboratory at different times or in different circumstances. Dietary variations that can alter the response to carcinogens include the total caloric intake (Tannenbaum and Silverstone, 1957), the level of nutritional components such as total fats, specific dietary substances (for example, vitamin A) or the presence of dietary contaminants such as antioxidants.

An important source of variability is the intrinsic or induced difference in

absorption, distribution, metabolic fate and excretion of carcinogens. Carcinogen concentration at target cells can be markedly affected by such differences, and it is therefore important that they be considered in quantitative analysis of the results. Clearly, if a compound is not significantly absorbed by a given test organism, no response does not indicate that it is not carcinogenic in other organisms that can effectively absorb and/or metabolize this compound.

Quantitative methods have been extensively used to study the metabolic pathways and kinetics of different carcinogens in several animal species and in human tissues. Wide inter-individual quantitative variations in the metabolic activation rate of carcinogens have been found, especially in those species that are genetically more heterogeneous, including the human species.

Assumptions of equal susceptibility of an experimental species and the human species that are made for risk assessment purposes on the basis of data obtained in a group of test animals (especially if inbred), are highly questionable (1) because the animal test group (representing a particular strain in a particular set of test conditions) is likely to differ widely in its response from other groups and strains of the same species; and (2) because the human populations are composed of individuals that may widely differ in their susceptibility to chemicals. No single species or strain or set of test conditions is the 'most susceptible' to each compound out of a range of carcinogens of different types with different metabolic requirements and different organotropisms. In the presence of data from only two or a few test groups out of a wide range of possible test conditions, assumptions about the 'most susceptible' species are unlikely to be generally valid.

Quantification of Dose Risk assessments are often made for human exposures to carcinogens that may have a wide variety of patterns. Examples include drugs prescribed for intake at discrete intervals during short periods of time; occupational exposures occurring only for a certain number of hours, 5 or 6 days per week, for a certain period of the adult life of the subject; and general environmental contaminants which may affect an individual from preconceptional and intrauterine exposures throughout development and adult life. Analogous variations occur in the exposure patterns of experimental animals, ranging from single exposures to continuous exposures, and which may be initiated at adult age, at birth or through the parent generation.

Dose schedule is known to affect cancer response in animal assays quantitatively, and sometimes qualitatively as well. Fractionated doses are often more effective than the corresponding single doses, partly because of different rates of metabolic utilization. In long-term animal assays, a daily dose (for example, in the diet) may be continued long after the time when tumours are induced, and the dose needed to induce the cancer may thus be overestimated. Needless to say, quantitation of dose is of particular importance in quantitative studies of dose–response relationships and for risk estimation; therefore, methods used for dose

determination need to be critically selected and clearly reported. Examples of quantitation problems include level of impurities, amount of spillage, stability of test compound, and frequency and type of monitoring procedures.

Target tissue dose estimation is very useful, if reliable methods are available. Critically evaluated procedures should be used for such estimations, or developed when necessary, to provide dose approximations that are more relevant for risk estimation than is the crude estimate of whole animal dose.

Quantification of Response Protocols for quantitative long-term carcinogenicity assays vary considerably in the choice of the time of observation of tumour response. Three main types of protocols have been used extensively:

(1) serial sacrifice protocols, giving a measure of tumour incidence at different times after the onset of treatment, but requiring large numbers of animals if the level of response is relatively low;
(2) terminal sacrifice protocols, providing quantitative comparisons at a standardized point in time, usually representing 75% or more of the predicted life-span of the animals, when a considerable proportion of control animals are still alive; and
(3) lifetime protocols with no scheduled sacrifice time, which allow all animals to be observed until moribund, and thus make it possible to detect late tumours.

Considerable differences in total tumour yield can be recorded following the same treatment, depending on the duration of the observation period.

Quantitation of results is also influenced by the tumour incidence observed in control animals, including historical colony controls as well as untreated and vehicle treated concurrent controls. Certain tumour types are usually found to occur with an easily detectable frequency in control groups (for example, in 5–25% of animals), and the variation of the frequency in different control groups gives an important measure of the background variability and of the significance of observed incidence in treated test groups. Other tumours occur less frequently and are often considered as 'rare' although an incidence of 0.5% for a specific tumour type would not be considered rare in a human population. When tumours occur in control groups with an incidence above 25%, the interpretation of quantitative response in treated groups often becomes difficult. Neoplastic response is measured by counting the number of tumours induced under a defined protocol, but such counts can be expressed in different ways (for example, total number of tumour bearing animals, total number of tumours, total number of tumours of a given type, number of tumours of a given type per animal, number of tumours observed during the life of the animal, number of tumours at death). The number of tumours observed is often a function of the extent and thoroughness of pathological examinations (for example, microscopic study of some organs or of multiple tissue sections).

The time at which tumours reach a size when they can be diagnosed is an

important variable. There are tumours whose time-to-occurrence is reduced although the rate of appearance and the final incidence are similar in the control and treated groups. Other tumours reach a higher final incidence because the rates of appearance in the treated groups are higher than in the control group. Tumour acceleration and tumour induction are terms used to describe these two phenomena. Tumour induction should be expressed as the probability of induction per animal exposed, as a function of time. Comparison of the resulting age-specific induction rates may allow the evaluation of the relative contribution of tumour induction and tumour acceleration to the total incidence. Other corrections, for example, for intercurrent or competing disease, must also be applied to reach a meaningful conclusion.

The evaluation of tumour response cannot be separated from the evaluation of biological nature of the induced tumours. This issue relates not only to the relative frequency in treated and control groups, but also to the similarity of experimental tumours to their human counterparts, and to the degree of malignancy expressed by the observed tumours. Diagnostic classification of malignancy in experimental animals is sometimes difficult and depends on painstaking search for histological evidence of invasion or metastasis or on biological studies of tumour transplantability. In some circumstances, benign tumours may kill animals through indirect mechanisms, and in certain cases malignant tumours may lack some characteristics of malignancy. The existence of species specificity renders the comparison of malignancy between different species even more difficult. Satisfactory models have not been developed to account for such biological differences in response, but it would appear useful to give a greater biological significance to, say, a 30 % increase in the incidence of carcinomas of the larynx, the bronchus, the colon or the pancreas in appropriate animals than to the induction of a comparable increase of commonly observed tumours such as skin tumours, hepatomas and lung adenomas in certain strains of mice or mammary tumours in certain strains of rats.

Models for long-term assay for carcinogenicity in animals have been progressively refined to provide healthy animal colonies free of intercurrent disease as much as possible. The human population, to which experimental results have to be transferred, includes, however, a variety of individuals who may be at high risk because of age, genetic defects, concurrent disease and concurrent exposures to other agents involved in cancer causation. This discrepancy has been well recognized in occupational epidemiology as the 'healthy worker effect'. It is suggested that assays for carcinogenicity of single compounds in healthy animals may involve a similar protective factor which could be called the 'healthy animal effect'.

Quantitative interpretation of long-term assays for carcinogenicity requires statistical methods for evaluating the significance of experimental results (see for example IARC, 1980). Mathematical models for cancer risk evaluation are discussed in section 3.2.3.

3.2.2.2 *Quantitative Comparisons of Data from Different Short-term Tests for Carcinogenicity*

Type of Tests Considered Since there is a great deal of uncertainty about the mechanisms of cancer induction, qualitative criteria derived from validation studies have been used to select the most appropriate tests for routine screening. In these validation studies, the presence or absence of carcinogenicity and of activity in short-term tests are compared and the accuracy of the test in its ability to predict carcinogenicity defined. On the basis of published validation studies the *Salmonella* plate incorporation assay (Ames *et al.*, 1975) is currently the only test considered to be fully established. From among the 100 or more short-term tests available, a few can be considered to be developed to the extent that they are now used as screening tests. These include induction of unscheduled DNA synthesis in mammalian cells, neoplastic transformation of mammalian cells in culture, induction of sex-linked recessive lethal mutations in *Drosophila* and induction of micronuclei in mice (Purchase, 1982). Many others are being developed.

Most of these tests are based on the induction of heritable damage to DNA, although in some cases precise definition of the mechanism involved is not possible.

Selection of Tests Validation studies provide a general view of the accuracy of tests. However, it has been found for all tests that the accuracy of tests for certain classes of chemicals is inadequate. Thus, even for qualitative assessment of carcinogenic potential it is advisable to select the most appropriate test system on the basis of knowledge of the chemical class under study. It is reasonable to anticipate that the same phenomenon will affect the quantitative comparability of results from short-term tests.

Metabolic Activation The importance of metabolic and kinetic factors in producing an active metabolite at the site of action has already been stressed. There are likely to be major differences in the metabolic and kinetic pathways between *in vitro* and *in vivo* assay systems. In particular, the balance of activation and detoxication (less detoxication *in vitro*), the proximity of the test material to the target site and the absence of excretion pathways *in vitro* have been shown to affect the qualitative results of short-term assays (Purchase, this volume). A similar impact on quantitative results is anticipated.

Many of these factors may be similar qualitatively (and sometimes quantitatively) in similar *in vitro* systems. Thus a greater degree of quantitative similarity may be expected between various similar *in vitro* systems than between results from *in vitro* and *in vivo* systems.

Qualitative Aspects Short-term tests rely on a variety of endpoints including

detection of phenotypic changes which cause alteration in growth characteristics and the presence of visible markers of genetic alterations. In most cases these are specifically selected to reflect a particular event (for example, mutation, chromosome damage or neoplastic transformation) and hence are not strictly comparable from a biological point of view.

For qualitative purposes various criteria have been applied to judge whether a positive or negative result is obtained. These are either empirical (for example, at least a doubling of colony counts) or based on statistical evaluation of differences in response in comparison to controls. Quantitative comparisons do not require qualitative judgments of this type.

Quantitative Aspects A clear dose–response relationship based on precise definition of the endpoint from a standardized test is the prerequisite for quantitative comparisons. For most of the assays widely used for screening there are precise endpoints leading to dose–response relationships. Some assays, notably those based on malignant transformation, have a variety of possible endpoints some of which are based on subjective criteria. Refinement of the criteria for reproducible, clearly defined endpoints are a prerequisite for accurate quantitative comparisons.

Many short-term tests are at an early stage of development and depend on a high level of skill for their execution. Standardization of tests, with careful study of factors influencing reproducibility, will assist in obtaining reliable data. Only a few such studies have been reported, predominantly for the *Salmonella* test (Dunkel, 1979; de Serres and Shelby, 1979).

Short-term tests can provide dose–response data for quantitative comparisons. However, in common with most biological assays, there may be a large degree of variability in results between tests run at different times and in different laboratories (see Bridges *et al.*, 1981). A full appreciation of quantitative similarities between tests should include indications of intratest variability.

Availability of Data The above discussion of comparisons between short-term tests is based on experience gained in qualitative validation and use of the tests. No extensive data are available which would allow a direct quantitative evaluation of the commonly used tests.

3.2.2.3 The Use of Potency Estimation from Short-term Tests to Predict Potency in Long-term Rodent Studies

Potency Estimation in Rodent Studies The many difficulties encountered in the conduct of whole-animal carcinogenicity studies and their impact on the assessment of potency are described in section 3.2.2.1. In comparison with short-term tests, the particular features include dosimetry, metabolism and kinetics,

qualitative aspects of judging cancer response and differences in mechanism of action.

Potency Estimation in Short-term Studies The main factors likely to have an impact on potency in short-term tests are dosimetry, metabolism and kinetics, and mechanism of action. The particular features of concern when comparisons with long-term rodent studies are envisaged include dosimetry, the nature and rate of metabolic conversion, variability in response and the mechanism of action.

(1) *Dosimetry*. Simple numerical comparison of daily doses in lifetime studies and the single dose in short-term tests glosses over the conceptual difficulty of equating these estimations, the lifetime studies incorporating a time function absent in short-term tests.

(2) *Metabolism, Kinetics and Target Tissue Dose*. For many carcinogens extensive metabolic activation is a prerequisite for producing a chemically reactive species in the target cell. Artifacts imposed by cell disruption required to provide metabolic capability in many *in vitro* systems means that the rate of formation and in some cases the structure of the reactive metabolite differs substantially from *in vivo* systems. The absence of excretion and an increase in the activation/detoxication ratio contribute to these differences. The ratio between applied dose and target tissue dose is likely to differ between the systems particularly at different doses.

(3) *Mechanism of Action*. The mechanism of carcinogenic action is not well understood but enough is known to suggest that there may be substantial differences from mechanisms acting in short-term tests. These differences make it difficult to envisage similarities in the shape of dose–response relationships even if point estimates of potency seem similar.

Comparison of Data from the Salmonella Test with Carcinogenic Potency Nine published studies have been reviewed by Purchase (this volume). A wide variety of chemicals (including nitrosamines, polycyclic and heterocyclic hydrocarbons, acetylaminofluorene derivatives, direct acting alkylating agents and butter yellow derivatives) have been tested. In spite of the correlation suggested by Meselson and Russell (1977), none of the other studies has shown a good quantitative correlation between these two types of tests. Addition of data from 4 nitroso compounds originally omitted by Meselson and Russell reduced the correlation. Thus, using estimates of potency based on applied dose in a variety of laboratories, prediction of carcinogenic potency from short-term data is prone to substantial errors.

Comparison of Data from Mammalian Cell Mutation Assays with Carcinogenic Potency Two studies using point mutation in Chinese hamster and mouse lymphoma, respectively, have been reported. In the first, the most potent

carcinogen among the 10 polycyclic hydrocarbons tested was also the most potent in the Chinese hamster mutation assay. A reasonable relationship between mutagenic potency in mouse lymphoma and carcinogenic potency has been described (Clive *et al.*, 1979) although variations of up to 104 in potency (with an average of 10-fold) are included in the data.

Other Test Systems Few data are available on other test sytems. *In vivo* short-term tests in mice and *Drosophila* were able to provide a quantitative separation between the carcinogenic and putative non-carcinogenic analogues of pairs of structurally related compounds (Purchase *et al.*, 1981). This observation should encourage further exploration of *in vivo* short-term tests for potency estimation.

Utility of Short-term Test Data The remarkably good correlation between carcinogenicity and the results from short-term tests (particularly the *Salmonella* plate incorporation assay), at least for certain categories of chemicals, defines a place for short-term tests in screening chemicals for carcinogenicity. Quantitative comparisons between these data suggest that short-term tests are not suitable for predicting carcinogenic potency. The differences in mechanisms, dosimetry, metabolism and endpoints between the two systems help to explain this poor quantitative correlation. Better estimates of target dose may help to reduce the variability.

3.2.2.4 Conclusions

(1) Qualitative and quantitative differences in metabolism of carcinogens are a major source of quantitative differences in response seen between short-term tests, long-term animal studies and human observations.

(2) Since the quantitative relationship between the exposure concentration of a chemical and its chemical form and concentration at the target site is often not known, dosimetry based on target site concentration and the average amount of binding to cellular macromolecules per cell can provide a more realistic estimation of the effective dose.

(3) Methods for expressing dose in studies using prolonged exposure and comparing them with short-term tests have not satisfactorily resolved the issue of how to integrate dose with respect to time.

(4) Although short-term tests are useful in screening chemicals for potential carcinogenicity and/or mutagenicity, they have not been developed to a stage where satisfactory quantitative extrapolation to lifetime carcinogenicity can be achieved.

(5) Metabolic and dosimetric studies, short-term tests, long-term carcinogenicity studies, epidemiological evidence and mathematical modelling are all important aspects to be considered in risk assessment. Each of them has relevance to particular aspects but none of them can be relied upon in isolation.

Thus the development of estimates of risk for practical purposes is a comprehensive evaluation which draws elements of judgment from all available toxicological and epidemiological information.

3.2.2.5 Recommendations

(1) As quantitative extrapolation from the different biological systems used for carcinogenicity testing depends on accurate estimation of effective dose, further research is needed to improve methods and enlarge the data base for comparative metabolism and for estimation of target tissue dose.

(2) In cancer risk analysis, each major type of neoplasm should be considered separately.

(3) Certain features of the design and conduct of long-term carcinogenicity studies are critical for accurate quantitative risk analysis. In this respect, particular attention should be paid to a careful pathological description of each animal, its age at death and its cause of death.

(4) There is an urgent need for further development of quantitative aspects of short-term tests, and studies are needed on the quantitative relationship between these tests and long-term bioassays.

3.2.3 Epidemiology, Statistics and Mathematical Modelling*

In the present state of biological knowledge, it is mainly through epidemiological studies that the human health hazard of a particular chemical can be decisively determined. The purpose of this section is to describe briefly the various types of epidemiological studies, their strengths and weaknesses, the data required for quantitative estimation of risk in humans in relation to dose and time characteristics of the exposure (dose–time–response relationships), and the methodological standards which must apply if any confidence is to be placed in the results. Detailed consideration will be given to methods of analysing dose–time–response relationships in epidemiological studies, particularly within the framework of models of carcinogenesis, and of the way in which measurement errors and the selection of an incorrect model may affect the results. The extrapolation from the observed data to exposure levels and time characteristics of response which are outside the range of observation, but necessary for the setting of exposure limits, will then be considered.

The word risk is used repeatedly throughout the document. In this section it will be used in its narrow technical sense to mean the probability of developing a disease. In some passages it may mean attributable risk, that is, the increase in risk contributed by a particular exposure, and in others the term relative risk may

* Sections 3.2.3, 3.2.4, 3.2.5 and 3.2.6 were prepared by B. Armstrong (Chairman), R. E. Albert, E. Anderson, K. P. Cantor, N. Day, D. G. Hoel, N. Ja. Janyseva, E. Komarov, Ju. I. Kundiev, A. Massoud, J. V. Neel, B. Ordóñez, J. Peto and Sir Edward Pochin.

be used, i.e. the ratio of the risk in individuals exposed to an agent to the risk in individuals not exposed. In practical terms, risk in humans is always derived from the incidence rate of disease as determined in epidemiological studies.

Although epidemiological methods are applicable to the study of all types of effects of environmental chemicals, both the epidemiological and mathematical and statistical discussions in this section strongly emphasize the experience acquired in the study of chemical carcinogens.

3.2.3.1 Estimation of Dose–Time–Response Relationships from Epidemiological Studies

While epidemiology may be the only method for quantifying late effects of chemicals in human subjects, it suffers from several obvious limitations. The following are the most important:

(1) Most epidemiological studies are based on historical exposure data which are often recorded inadequately or are subject to the uncertainties of human memory. Thus, adequate estimation of the dose of a chemical may be difficult if not impossible and the data on potential confounding or interacting factors may be equally deficient.

(2) Regardless of the quality of exposure information, in most studies direct risk estimates will be possible only for those levels and periods of exposure which have affected a substantial number of human subjects. Outside of these observed ranges of time and exposure levels, extrapolation will be necessary if risk estimates are to be made.

(3) Exposure to several chemicals is the rule rather than the exception, and it is very difficult in a single study to implicate one chemical as the cause of an effect unless high quality data on individual exposures to each relevant chemical are available; this is rarely the case. Even in multiple studies, unless there is substantial variation in the mix of chemicals present, it may still be difficult to determine which chemical, if only one, is responsible for an observed effect.

(4) For precision in estimation of risk, large numbers of study subjects will usually be required, particularly if the exposure is low or to a rare chemical, and the increment in disease caused by the chemical is small in comparison with the baseline rate. Even if the numbers are sufficient to detect an overall increase in risk, they will be almost always insufficient for precise modelling of dose–time–response relationships and the nature of any interactions. In quantitative terms, exposures which lead to relative risks of less than 1.2 are unlikely to be identifiable unambiguously as cancer risk factors. For most human cancers a relative risk of 1.2 translates into an increase in absolute lifetime risk of between 1 in 100 and 1 in 1000. These figures should be contrasted with levels of 1 in 100 000 and 1 in 1000 000 to which extrapolation is required, indicating the size of the gap between observable levels and those which may be required for regulatory purposes.

(5) It is a truism that epidemiology can say nothing about the effects of chemicals unless human exposure has occurred and health effects have been observed. Thus, while epidemiological surveillance may be mounted to give the earliest possible warning of a new adverse effect, it cannot prevent the introduction into use of new chemical agents except through the analogy that may be drawn between the observed effects of one chemical and the likely effects of another.

(6) These limitations mean that many, if not most, decisions on permissible levels of human exposure to chemicals will have to be taken in absence of, or with inadequate epidemiological data. One may ask then: Why do epidemiological studies at all? There are at least two persuasive answers:

(a) Chemical hazards have not always become obvious through studies in other biological systems before health effects have occurred, and not all will necessarily be predicted in this way in the future. Thus epidemiology has an important role to play in the continuing detection and control of chemical hazards.
(b) More important to the subject of this report is that detailed quantitative study of the hazard of chemicals in humans will permit, through comparison with responses in other biological systems, a greater confidence in or, at least, a better understanding of the use of these systems for predicting the likely response in humans. The effects of chemicals vary widely between animals, and the correspondence, particularly in carcinogenesis, between the results of animal experiments and the responses in other biological systems is poorly established. Laboratory studies are useful for the detection of potentially toxic agents but their use as a basis for quantitative risk assessment is uncertain in the absence of human data on similar agents.

Assessment of Genetic Effects This section of the report deals mainly with the epidemiology of chemical carcinogenesis, rather than mutagenesis and other effects of chemicals. There is, however, an almost equal interest in the possible mutagenicity as to the possible carcinogenicity of the various chemicals to which human subjects are exposed.

The fact that there are so many chemical carcinogens and mutagens raises the question of an increase in heritable mutations in the germinal tissue of the exposed population. The possible impact of an increased mutation rate on the human population has been extensively treated in the various reports of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 1977, 1982).

To date there have been no studies seeking evidence of an increased mutation rate in populations exposed to a chemical mutagen. When such studies are undertaken, all the methodological and epidemiological problems mentioned in

the following sections arise as do several additional problems (see Neel *et al.*, this volume). In particular, the most suitable indicators of an increased mutation rate either are not collected through public health statistics or are collected rather poorly (as in the case of certain sentinel phenotypes unequivocally due to mutation). This imposes the requirement of rather elaborately designed special studies, necessitating extremely large numbers of observations. The most objective and the least equivocal indicator of an elevated mutation rate at present would be an increase in chromosome abnormalities and variant proteins present in the child of exposed parents but not in the parents themselves. Current technical developments should make the collection of both such types of data less laborious and expensive in the future (see Neel *et al.*, this volume).

The choice of human populations for the study of chemical mutagenesis is difficult. Unlike for radiation exposure, one cannot readily estimate gonadal dose from ingestion of or from external exposure to a chemical, especially when the exposure occurred some years previously. Even when a definitely high risk population, such as children of exposed individuals, has been identified, it is unlikely by itself to supply the number of observations necessary for unequivocal demonstration of increases in mutation rates such as should not go undetected. Under these circumstances, it is suggested that in the early stages of studies of human chemical mutagenesis attention should be concentrated on the highest risk populations that can be identified. These populations and studies will be dispersed throughout many countries, thus requiring the development of a set of standard guidelines, so that the result of studies carried out in different countries can be readily compared and combined. Candidate populations include the children of individuals exposed to unusual concentrations of pesticides, of persons who have survived (with treatment) childhood cancer, and of persons who have developed certain occupational cancers.

Estimation of Cancer Risk Common themes that underlie consideration of all epidemiological studies are sources of data, sample size, confounding and bias, estimates of exposure, data quality, and inferences from study findings. The types of studies to be discussed are studies of demographic correlation, case-control studies, cohort studies, and field trials of preventive agents or environmental control practices.

(1) *Studies of Demographic Correlation.* In these studies (sometimes called 'ecological' studies) the unit of study is a population or a population sample, and the average population exposure to the agent under study is related to the aggregate disease experience, as measured by incidence or mortality data. The primary examples are studies of geographical correlation in which the geographical distribution of cancer rates is associated with the distribution of environmental factors or industrial characteristics; and time–trend analyses that relate temporal changes in cancer rates with previous increases or decreases in population exposure rates.

When it was first suggested in the United States that byproducts of water chlorination might be causally associated with cancer, several geographic correlation studies were initiated as to which anatomic sites might be implicated (see Cantor, this volume). These studies served as hypothesis-generating exercises and pointed to bladder, colon and rectal cancers as deserving further evaluation. Studies of cancer rates in Japanese migrants to the United States suggested that stomach cancer, which remained high among persons migrating after age 15, is caused by an 'early-stage' carcinogen; whereas colon cancer, with rates that increased from the low Japanese levels regardless of age at migration, is probably related to a 'late-stage' factor (Haenszel, 1961; Haenszel *et al.*, 1973). An international comparison of *per caput* animal protein ingestion and colon cancer rates pointed to strong dietary influences (Armstrong and Doll, 1975). In these cases, results of correlation studies served to generate or refine hypotheses about causes of cancer or mechanisms of action that then required further elaboration.

The temporal increases in lung cancer among men that followed, with a 20-year delay, increases in male smoking rates, is now being mimicked by similar observations among females whose smoking rates increased more recently (Doll and Peto, 1981). These observations are in concordance with results from an extensive body of analytical epidemiology relating cigarette smoking to lung cancer and are fully supportive of the hypothesis. 'Epidemiological consistency' with analytical results was also observed in temporal patterns of endometrial cancer in the United States, which rose and fell with the earlier increase and decrease in post-menopausal oestrogen use (Jick *et al.*, 1980). Correlation studies in these settings substantiated, and thereby gave additional weight to, results from other types of epidemiological studies.

The strength of correlational studies is in their use of existing data bases which usually enable rapid completion of studies at relatively low cost. Correlation studies, however, are not able by themselves to resolve the question of causality, although in some circumstances they can provide strong supportive evidence. One rare instance might be the geographical association of intake of aflatoxin in food with primary liver cancer (Linsell and Peers, 1977), especially if it can be shown that reducing aflatoxin intake reduces the incidence of primary liver cancer. In most other situations, the likelihood of inadequate definition or misspecification of exposures and the presence of unknown or unexpected confounding factors is such that the major contribution of correlation studies is not to estimate quantitative risk but rather to generate hypotheses and provide supporting evidence to other epidemiological data.

(2) *Analytical Studies.* In contrast to studies of demographic correlation, in analytical studies the unit of analysis is the individual. Analytical studies seek associations between individual exposures or host characteristics and disease experience. Measures of association are expressed as estimates of relative risk and as such they may be used directly in risk analyses. Extrapolations of risk

estimates to predict the risk associated with exposures outside the range of observations depend on the mathematical models used (see sections 3.2.3.3 and 3.2.3.4). Two types of analytical studies most commonly used are cohort studies and case-control studies.

Advances in techniques for measuring biochemical and cytogenetic indicators of exposure, nutrition status or genetic susceptibility will find increasing application in both cohort and case-control studies. Opportunities to gather such data should be exploited where there is good scientific rationale, because such measures can furnish important links between the epidemiological observations and independent experimental laboratory evidence of genetic damage or cancer risk.

(a) *Cohort Studies.* Selection of a cohort for epidemiological study is generally determined by exposure, such as to occupational or environmental factors or to therapeutic treatment, for example, ionizing radiation (X-rays). A cohort study is the design of choice for evaluating effects of high level exposures to factors that are rare in the general population but common in identifiable subgroups. As such, it has been the favoured approach in occupational epidemiology. Exposure estimates in occupational cohort studies are often based on 'job-exposure matrices' (Hoar, 1982) that relate particular job titles from a person's work history within the industrial setting to measurements or to informed estimates of exposure levels to one or several agents. Inaccurate estimates within the job-exposure matrix, or incorrect listing of job titles for an individual will generally bias estimates of relative risk toward unity. Assessment in occupational cohort studies of ancillary exposures, such as to cigarette smoke, can be extremely important for the accurate assessment of risk, particularly if they interact with the chemical under study or are possible confounding factors.

Although the cohort approach finds its primary application in studying highly exposed occupational groups, there are special circumstances where samples from the general population have been or may be used. Exposure information on a variety of factors, including demographic features, occupation and smoking is already available for sizable numbers of persons who participated in studies of cardiovascular disease, national health surveys, or other special studies. Although the primary rationale for collecting these data was usually not the future measurement of cancer risk, follow-up of these general population cohorts may be quite valuable in providing such estimates.

(b) *Case-control Studies.* Inclusion of a subject into a case-control study is based not on exposure, but on disease status. Exposure to suspect agents among cases and in a matched series of non-diseased persons (controls) are determined retrospectively. Exposure ascertainment may come from direct interviews, from medical records, from linkage with occupational histories, or from other data sources. The case-control design is best used to study relatively rare diseases (such as most cancers) where exposure to the suspect causative agent is common in the general population (20–80% exposed). If there is a very strong association

between the cancer in question and the exposure (for example, angiosarcoma of the liver and vinyl chloride monomer; mesothelioma and asbestos), the exposure need not be so common, but these are unusual situations. Case-control studies are likely to play a bigger role in the future in estimating risks associated with exposure to chemicals. The focus of cancer epidemiology is turning from high occupational exposures to less intense occupational exposures, to exposures to agents found in the general environment, and to dietary factors; in these situations case-control studies are likely to be most efficient.

Large population-based case-control studies conducted in settings with a range of exposure possibilities can make important contributions to our understanding of the risk from long-term low-level exposure to environmental contaminants in the ambient air, in drinking water and in food. Care must be taken in such studies to collect detailed information on all factors that may be relevant to cancer risk, so that interactions and dose–response relationships may be properly evaluated. Large samples are required, not only to allow detection of relatively low risks, but also to permit analysis of risk within selected subgroups.

(c) *Intervention Studies.* Confirmation of the validity of epidemiological observations from cohort or case-control studies can come from field trials of putative preventive agents and from implementation of regulatory or other measures to control occupational or environmental exposures.

(3) *Design Requirements for Epidemiological Studies.* In general terms, for an analytical epidemiological study to achieve its objectives the requirements listed below should ideally be met. Failing this, the best available data, carefully analysed, will usually be better than no data at all. The requirements are:

(a) inclusion of sufficient study subjects and sufficient duration of follow-up after initial exposure to the chemical to detect the lowest excess risk considered important, according to an appropriate model of the time relationships of response;
(b) valid and quantitative estimation of exposure to the chemical. In quantitative evaluation of dose–time–response relationships, quantitative estimation of exposure is particularly important, and should include dose rate characteristics of dose delivery (for example, whether continuous or intermittent), time of the first exposure, time of the last exposure and total duration of exposure;
(c) identification, quantitation and control, in design or analysis, of all relevant confounding factors including other chemicals in the workplace (when an industrial chemical is under study) and life-style factors which may be relevant to the disease in question;
(d) quantitative measurement of other risk factors which may interact with the chemical under study;
(e) minimization, where possible, and otherwise identification, description and control of factors in study design (or in the study situation) which may bias the estimate of relative risk associated with the exposure.

A more detailed treatment of the problems of bias and of confounding and interacting factors in epidemiological studies can be found in Breslow and Day (1980) and Monson (1980).

3.2.3.2 Dose–Response Models

For purposes of risk estimation, mathematical models have been constructed to describe quantitatively the dose–response relationships in laboratory experiments and epidemiological studies. Various models are generally consistent in providing equivalent numerical estimates of the probability of effect within the range of experimental observations. They do, however, begin to seriously disagree in many applications when estimates of the effect are made at dose levels or time points well beyond the range of experimentation. This is one of the major scientific issues in risk estimation, since estimates of the effect are typically required at low dose levels. The choice of model or models is critical and must be made on the basis of biological reality. The choice among models generally cannot be made only on the basis of experimental data because most of the proposed models are sufficiently flexible so that they will all adequately describe the experimental data.

It is believed that the Armitage–Doll (1961) multistage model is the most reasonable mathematical description currently available. The important implication of applying this model to low-dose risk estimation is that it is essentially linear at low doses. Linear procedures have been criticized in general for being overly conservative. It has been shown quite generally (Crump *et al.*, 1976) that in those instances when the carcinogen acts in an additive fashion with respect to the spontaneous background tumours, the dose–response function is linear at low doses. Furthermore, in many cases only a small portion of the background need be additive for this to be true (Hoel, 1980). Thus linearity may not be as conservative as it is believed to be. The multistage model and most others ignore the issues of the difference between applied and effective dose. The kinetics relating administered to 'effective' dose may need to be incorporated into the model. In many cases simple first-order kinetics is all that is involved, in which case no transformation of dose is required. In other situations saturation of detoxication and repair systems may cause non-linearities in the dose transformation function. This in turn may produce a threshold-like dose–response relationship. Therefore, there is a need to obtain biochemical kinetic information to supplement the basic applied dose and tumour response data prior to statistical model fitting (Hoel *et al.*, 1983).

It should be recognized that all of the available methods for extrapolation of results down to low doses do not provide a useful extrapolation between species. Some of the variables with respect to applied dose can be incorporated as mentioned above. No method is available for incorporating differing susceptibilities of the experimental species and man, unless experimental data and

epidemiological data are available for comparison. Hence, the results of such low-dose extrapolation calculations may be misleading in that they cannot incorporate an important variable.

The use of safety factors or acceptable daily intakes (ADIs) is an entirely different process from risk estimation using mathematical models. The main difference is that an ADI procedure is a method for setting exposure standards and thus indirectly includes a value judgment concerning acceptable risk. The mathematical approaches on the other hand make no judgments but instead simply attempt to estimate an effect at a given dose. The use of ADIs in situations where thresholds are not necessarily present is difficult because there is little or no understanding of the actual risk at the ADI level. The advantage of its use is that its very crudeness indicates that little is known. Unfortunately, a possibly false sense of safety or security may be associated with the application of ADIs.

Quantitative comparisons between various short-term tests and long-term whole-animal studies require the use of mathematical models. The objective is to reduce the results of an assay of a particular compound to a single number which measures potency in some sense. For example, statistical methods are available (Sawyer *et al.*, 1983) to estimate that dose level in an animal carcinogenesis study which would produce 50% tumours in a long-term study of animals which are normally tumour-free. The mathematics for this procedure requires a model which incorporates both dose and time (for instance, multistage model). Such a model is needed, for example, with experimental data obtained in tests of less than lifetime duration. Other statistical models not requiring a time component have been created for potency estimation in short-term tests such as the Ames *Salmonella* test (Bernstein *et al.*, 1981). Although potency estimation often uses the same somewhat speculative mathematical models as are used in low-dose risk estimation, the procedure does not become involved with the scientifically questionable process of low-dose estimation.

3.2.3.3 The Use of Different Models of Carcinogenesis

The two variables which are of major importance in determining risk, and which have to be incorporated in any relationship associating exposure with risk, are time and dose. Both these variables, however, are complex. From both experimental and epidemiological data it has become clear that several aspects of time need to be considered, including time since exposure started, time since exposure ceased, duration of exposure and age. Similarly, 'dose' may be expressed as total dose, average daily dose, maximum daily dose and so on. If dose–time–response relationships are to be estimated from data which are unlikely to be very extensive, experience from other areas of carcinogenesis and toxicology has to be used in order to characterize 'time' and 'dose' in ways which are biologically justified and reduce the problem to a manageable form. This

experience is most readily expressed in terms of general models of carcinogenic action.

Experimental studies have demonstrated the multistage nature of carcinogenesis in terms of initiation and promotion for a number of sites of which the skin, liver, bladder and mammary gland provide the most explicit examples. Epidemiological data suggest that much of human carcinogenesis is also a multistage process. In this section we consider the consequences of such models for the treatment of 'time' and 'dose' in dose–time–response relationships.

In multistage models it is assumed that a cancer arises from a single cell which has undergone a series of changes, at least some of which take place in a specific sequence. These changes consist of transitions from one stage to the next, and agents which increase the cancer risk do so by increasing the rate at which one or more of these transitions occur. The details of these models can be specified in several ways, but in general terms the different specifications lead to similar predictions for dose–time–response relationships. All that is required is that some transitions may be termed early-stage transitions and some late-stage transitions. Other models have been proposed, some of which fit at least part of the available data reasonably well. These models may give rise to very different predictions of the risk at dose levels below the observable range. The reasons these models are considered less satisfactory than the multistage formulation adopted here are basically biological reasons. A large body of experimental data has accumulated which demonstrates that carcinogenesis is a process involving a succession of cellular changes. Any model of carcinogenic process should incorporate this multistage sequential aspect.

Continuous exposure at a constant level will be considered first. In this case, after exposure of duration t at a dose rate d, the incidence I of a specific tumour type will be given by a function of the form:

$$I(t, d) = \mathrm{g}(d)\mathrm{h}(t) \tag{3.1}$$

where $\mathrm{g}(d)$ is a function only of dose rate and $\mathrm{h}(t)$ a function only of time, both perhaps different for different tumour types. That risk can be factorized—a consequence of the multistage model and confirmed by both experimental and epidemiological observations—into a dose term and a time term has important consequences:

(1) The measure of dose of critical importance is the dose rate. If one uses in an expression for risk an alternative measure of dose, such as the total dose, the model may give incorrect results.
(2) The same function of time is appropriate for different levels of dose rate. This independence of the effect of time and dose invalidates several of the uses to which the concept of latent period has been put, or even the way it is defined (see Peto, this volume). In particular, the idea that latency increases with

decreasing dose to the point where no tumours are to be expected within the normal lifetime, is not supported.

In most multistage models the functions g(d) and h(t) in the expression for incidence are well approximated by simple power functions:

$$I(d, t) = kd^{\alpha} t^{\beta} \tag{3.2}$$

This expression provides an excellent fit to most data on continuous exposure to chemicals with α typically between 1 and 2 and β about 3–5. Fitting power function models of this type can be of great value in expressing the basic risk relationships underlying extensive sets of data. The resulting dose–time–response estimates are succinct descriptions of risk, certainly of use for interpolation within the range of observations.

With exposure of limited duration, a greater diversity is seen in the way risk evolves with time, as indicated in Figure 3.3. Algebraic expressions for the possible ways in which risk evolves can be complex, but there are two basic types of behaviour, corresponding to whether the principal mode of action of the agent is early-stage or late-stage. Description of the observed evolution of risk after exposure has been discontinued can be given, at least qualitatively, in terms of a multistage mechanism, even though no precise functional form is used.

When the exposure acts in conjunction with other factors, the age at which exposure begins is of some importance. The effect of age at which exposure begins

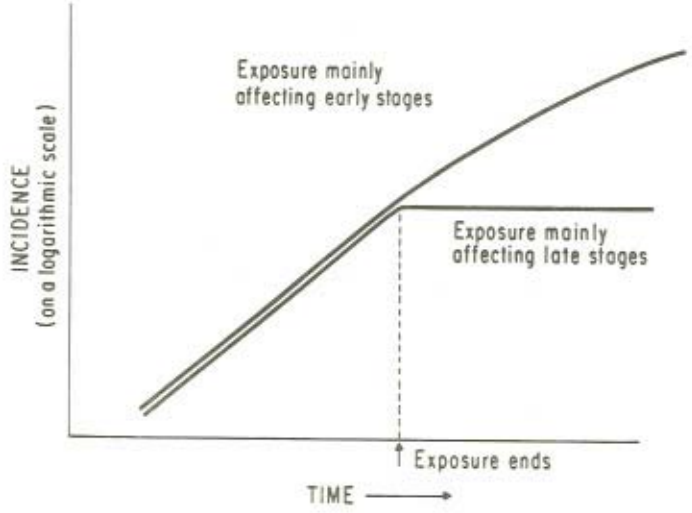

Figure 3.3 Tumour incidence for limited carcinogen exposure. Incidence evolves in different way with time, depending whether exposure affects early or late stages

on the subsequent risk depends on the stage at which the chemical primarily acts (see Figure 3.4). Exposure to chemicals which act mainly at a late stage will have a rapidly increasing absolute effect with increasing age at the beginning of exposure. Agents acting at an early stage will have a roughly constant absolute effect. The algebraic form for expressing the effect of age at the beginning of exposure may be complex but can be incorporated in a multistage model, at least in a qualitative way.

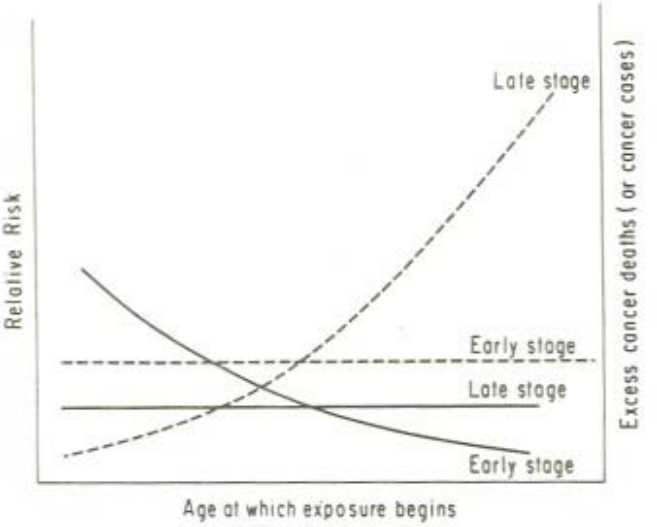

Figure 3.4 The effect of age at which exposure begins on subsequent risk. Interrupted line: relative risk; full line: excess cancer deaths (or cancer cases)

In expressions (3.1) and (3.2) given above, the tacit assumption has been made that most of the tumours observed are due to the exposure under consideration. This assumption may be adequate at high-dose levels, or in experimental situations where spontaneous tumous are rare. At lower dose levels and when tumours occur in the exposed population as a result of other causes, modifications are required. In expression (3.2), the rate at which the transition induced by the exposure occurs is taken as directly proportional to the dose. If two such transitions are exposure dependent, then the rate of their joint occurrence is proportional to the square of the dose, and so on. If, however, these transitions can occur spontaneously or as a result of other causes, then one might assume, as an approximation, that the rate of occurrence of an exposure-dependent transition is the sum of a background level and a level proportional to dose. The rate of this transition would then be given by $a + b \cdot d$, where a is the background, d the dose rate and b the transition rate per unit dose rate.

If several transitions depend on the exposure, then the rate of occurrence is the product of the rate of occurrence of each transition independently and can be written as $\pi\ (a_i + b_i d)$ (see Anderson, and Hoel, both in this volume).

This expression should replace the power function d^s in expression (3.2), so that the overall expression for incidence then takes the form

$$I(d, t) = \pi(a_i + b_i d)t^s \tag{3.3}$$

This form (3.3) would be more appropriate at dose levels at which the increase in risk is small.

3.2.3.4 Low-dose Extrapolation and Risk Assessment

It is often not possible to estimate the relative or absolute excess risk to man at any exposure level, much less to discriminate between models, but it may be none the less useful to discuss the principles underlying low-dose extrapolation. Uncertainty of the form of the dose, age and time dependence, sampling errors in the observed excess risk and inaccuracies in estimates of exposure level all contribute to the error, whether random or systematic, in the predicted risk at low exposure levels. The confidence that can be placed in such a prediction cannot be assessed even qualitatively unless the relative importance of these factors and their likely effects are considered individually. For example, the age and time dependence of cancers caused by asbestos are known, at least approximately, with reasonable confidence (Peto *et al.*, 1982), but available exposure data are poor and better exposure data and better information on the relative carcinogenicity of different fibre sizes are needed before reliable dose-specific risk predictions can be made for this agent. In contrast, data on patients treated with various alkylating agents may soon give quite precise estimates of the cancer risk caused within a decade or so of first exposure to accurately known doses. The longer term pattern of excess risk will, however, not be known for many years, and the reliability of any prediction will depend largely on the plausibility of this aspect of the assumed model; it will have to be inferred by analogy with the observed effects of other agents on man or from animal experiments.

Selecting a Model for Age and Time Dependence as a Basis for Extrapolation The most plausible multistage model should in principle be chosen to describe the likely effects of age, time and duration of exposure. Whether a single model can be selected with any confidence will depend on the availability of human and animal data on similar cancers and carcinogens, however, and if various models seem scientifically acceptable, the predictions should be calculated for each.

The importance of predicting the time dependence correctly is illustrated by the marked difference, after discontinuing exposure, between excess lung cancer incidence in cigarette smokers, which remains approximately constant after

exposure has ceased, and the incidence of mesothelioma following asbestos exposure, which continues to rise as the third or higher power of time. The life-long lung cancer risk caused by smoking for 15 years and then stopping may be more than an order of magnitude lower than that of a life-long smoker (Doll, 1978); but the mesothelioma risk caused by 15 years' exposure to asbestos may well be almost as high as that caused by life-long asbestos exposure at the same level.

The example of asbestos also illustrates the importance of age at first exposure and the fact that the same agent may act quite differently for different cancers. Asbestos exposure appears to multiply the lung cancer risk caused by smoking and the subsequent absolute risk increases with age and cigarette consumption but is not strongly dependent on time since first exposure. For mesothelioma, however, the risk depends strongly on time since first exposure to asbestos, but is hardly affected by age or smoking. This explains, at least qualitatively, the marked variation in the ratio of mesothelioma incidence to the absolute excess of lung cancer observed among insulation workers (Peto *et al.*, 1982). This ratio fell as age of exposure to asbestos increased, from about 1:2 in smokers first exposed to asbestos at age 20 to about 1:4 in smokers first exposed to asbestos in the early middle age, but was an order of magnitude higher among non-smoking insulation workers, as their cancer risk was very low. These data illustrate the qualitative assessment of model-dependent error. The roughly two-fold effect of moderate variation in age at first exposure to asbestos among industrial workers may not be considered very important; but the error would be unacceptably large if such data were used without correction for smoking to predict lung cancer incidence in non-smokers heavily exposed to asbestos among whom mesothelioma may be 3 or 4 times as common as lung cancer.

Selecting a Dose–Response Model Two types of dose–response models will be briefly considered here, linear and mixed linear–quadratic.

(1) *Assumption of Linear Dose–Response.* The dose–response for chemical carcinogenesis may often be roughly linear, but at least three human cancers for which dose–response data are available (leukaemia following ionizing radiation, lung cancer in smokers, and oesophageal cancer related to alcohol consumption) do not obey such a relationship (Tuyns *et al.*, 1977; Peto, this volume). The non–linearity of leukaemia risk caused by ionizing radiation may well be due to cell-killing, and it is reassuring that for these other two examples, and for several animal cancers, the exponent of dose appears to be greater than unity. The assumption of linear dose–response would therefore tend to overestimate the risk at low doses for these carcinogens. On the other hand, there are examples in other animal studies of sublinear dose dependence (Peto, this volume), and linear extrapolation may substantially underestimate the low-dose risk for some human carcinogens. The assumption of linear dose–response seems a sensible and

practicable convention on which to base risk estimation for regulatory purposes, but it cannot be said to be uniformly conservative.

Exposure estimates for industrial carcinogens are usually very inaccurate, and this large error should be taken account of explicitly in any dose–response analysis. The linear relationship, risk = $a \cdot \text{dose} + b + \text{error}$, of classical linear regression yields unbiased estimates, $\hat{a}$ and $\hat{b}$, of the coefficient of dose, a, and of the risk at zero dose, b, but large errors in dose will flatten the regression line, reducing $\hat{a}$ and increasing $\hat{b}$. The 'multistage' equation $I \propto (\text{dose})^a$ can be written: $\log I = a \cdot \log(\text{dose}) + b$, and the exponent of dose, a, will therefore be underestimated in the same way if doses are estimated inaccurately. Thus, for example, lung cancer incidence formula in continuing smokers appears to be approximately proportional to daily cigarette consumption in several studies, but the most accurate data suggest significant upward curvature (Doll and Peto, 1978). Apparently linear relationships may therefore in fact be quadratic, and apparently sublinear relationships linear.

(2) *Variation in Susceptibility.* Substantial variation in susceptibility to cancer induction has been demonstrated in apparently similar animals and may also occur in man. One effect of such variation is to reduce the estimated exponent of dose in the incidence equation, as at low doses the effect of variable susceptibility is similar to the effect discussed above of randomly varying the dose at each nominally similar dose level. In high-dose experiments, the exponent of time may also be reduced, as the most susceptible animals are eliminated, and the overall incidence at older ages falls progressively below the true curve. Parrish (1981) has shown that a true power of time of about 5 may well have been reduced to 3 by this effect in experiments in which mice were painted with benzo(a)pyrene. This might also account, at least in part, for the reduced susceptibility to promotion in old age observed in initiation–promotion experiments.

(3) *Mixed Linear and Quadratic Response.* The theoretical formulation of dose dependence for cancers that also occur naturally, outlined in section 3.2.3.3, implies that apparently quadratic dose–response curves are likely to contain a substantial linear component. This may not be detectable statistically, however, as in the observed dose range the quadratic term may dominate, and in any case a linear component could be an artifact due to the effects of dosimetry errors discussed above. Such a linear component would, however, have profound implications for low-dose extrapolation. Suppose, for example, that the risk of lung cancer due to smoking were proportional to $(1 + \text{constant} \times \text{dose})^2$ where d is (number of cigarettes per day) × 0.2. The difference between this model and a purely quadratic excess risk is shown in the following table (RR = relative risk; d = cigarettes/day × 0.2).

The models do not differ greatly in the observed range, but at low doses the quadratic model underestimates the risk by several orders of magnitude. For low-dose extrapolation, therefore, the largest linear component in a linear and quadratic mixed model consistent with the data should be assumed, even if the

Cigarettes per day	$RR = (1+d)^2$ (mixed model)	$RR = 1+3d^2/2$ (quadratic model)
0	1	1
0.01	1.004	1.000 006
5	4	2.5
10	9	7
20	25	25

observed data appear perfectly quadratic. If the exposure data are so poor that there is little or no correlation between estimated dose and risk, a crude but effective device for fitting a linear model is simply to average the excess risk and dose estimates, weighting by the number of individuals at each dose level, and to draw a straight line through this point and the origin.

(4) *Inappropriate Dose and Response Measures.* The preceding examples illustrate some of the gross errors that can arise if the dose–response relationship is formulated incorrectly. Other common sources of error of which some examples are given by Peto (this volume) are the inappropriate combination of dose and time in a single 'total' or 'cumulative' dose index; the analysis of prevalence rather than incidence and the misuse of latent period, either as a measure of exposure or to infer the existence of a safe threshold.

3.2.4 Practical Applications

The use of quantitative risk estimates for regulatory purposes is a subject of debate and no consensus has been reached. Quantitative extrapolation of response data from experimental animals to man is particularly contentious. This section describes approaches that are commonly used. They should not be necessarily taken as models of good practice.

Most countries have adopted some form of legislation which mandates the protection of the public from exposure to toxic chemicals. Some legislation makes specific reference to carcinogens, some cover carcinogens in more general health provisions. These various authorities have been developed at different times and incorporate a variety of legislative approaches, especially with respect to risk assessment. For example, in the United States, some statutes require that health standards be technologically feasible, others require balancing risks against social and economic factors; and some are health based without regard to economic or technological feasibility. The role of risk assessment is different in these various approaches, and there is no uniform approach to risk assessment from the legislative standpoint.

Considerable uncertainties are involved in the estimation of risk from exposure to suspect carcinogenic agents. Most often actual exposure data are unavailable

or incomplete. Therefore, estimates of exposure of human populations must generally rely on a variety of assumptions which generate many uncertainties. Considerable uncertainties are also involved in extrapolation from high doses in the observed response range to lower doses of interest to populations at risk. Additional uncertainties are involved in the extrapolation from animals to man; from full lifetime exposure studies to partial lifetime exposure; from long-term low-dose exposures to short-term high-dose exposures, etc. In short, there are uncertainties involved in both qualitative description of carcinogenic potential in the absence of human studies and in quantitative estimates of the magnitude of the public health problem associated with exposure to potential carcinogens. Because of compelling practical considerations underlying public health protection policies, quantitative risk estimation is needed in spite of its uncertainties. Recently, a comparative potency method has been used to estimate cancer risk (Harris, 1981; Albert, this volume). With this method, the estimation of cancer risk of a chemical under consideration is based on the risk for an established human carcinogen, taking into account their relative potencies as determined by *in vitro* and *in vivo* bioassay data. This approach has been found useful in estimating cancer risk from diesel engine particulate exhausts based on the lung cancer responses in humans associated with coke oven and roofing tar emissions and cigarette smoke (EPA, 1982).

The conventional toxicological approach to determining acceptable levels of exposure has been generally used in the Soviet Union for carcinogens (Janyseva *et al.*, this volume). The acceptable dose is determined by applying a safety factor to the highest dose which does not produce an observable response. The magnitude of the safety factor is arbitrary and takes into account the possibly greater average sensitivity of humans compared to animals and the extent to which some humans are more susceptible than the average individual.

Quantitative risk estimations, and in some cases safety factor approaches, have been used to set permissible levels of exposure for a variety of circumstances involving protection of workers, food safety, drinking water safety, pesticide residues in food and environmental releases of suspect carcinogens to the ambient air and water.

In other circumstances, where there has been inadvertent exposure of population segments to potential carcinogens, quantitative risk estimation has provided information regarding the urgency of a public health problem. Examples include risk to populations associated with releases of potential carcinogens from uncontrolled waste disposal sites, exposures associated with the use of urea–formaldehyde foam in home insulations and the projected public health burden associated with potential widespread introduction of diesel engines in motor vehicles.

Where there is a need to balance risks against social and economic concerns in deciding public health policies, quantitative risk estimation has been used to provide rough measures of hazard. For example, such balancing decisions are

required under two statutes in the United States: those involving the use of pesticides (FIFRA, 1978) and toxic substances (TSCA, 1977).

For technology based standards, quantitative risk estimation has been used to estimate the residual risk after application of best available technology; should the residual risk remain relatively high, technology forcing standards may have to be set.

Finally, there has been a practical need to set priorities for regulatory consideration involving a large number of potential carcinogens, as is the case for ambient air. Relative carcinogenic potency, together with the strength of biomedical evidence indicating carcinogenic potential and information about levels of exposure, have been used to set regulatory priorities. Also, such stratification of hazardous potential is being invoked to prescribe the most appropriate disposal techniques for hazardous waste, clean-up of toxic spills and the extent of clean-up for toxic waste sites.

In summary, a range of practical considerations require information about the extent of risk. Large uncertainties are involved in currently available risk assessment techniques. Until more is known about the mechanisms of carcinogenesis, these uncertainties cannot be removed. In addition to inherent uncertainties in risk assessment approaches, data for evaluating risk associated with individual chemicals are often inadequate; nevertheless, public policy decisions must be made and should be made on the basis of the best available evidence.

3.2.5 Conclusions

(1) Sufficient data for a complete quantitative modelling of the late effects of chemicals in humans in relation to dose and time characteristics of exposure have been rarely available; if available, such data have often been limited by the inadequacy of recording or subjective recall.

(2) Most chemicals exert multiple biological effects many of which may be useful indicators of human health effects. Multiple endpoints have not been used sufficiently in large-scale epidemiological studies.

(3) Quantitative assessment of cancer risk requires extrapolation of human data below the range usually observed in epidemiological investigations such as those of occupationally exposed individuals. The extrapolation should be based on the most plausible multistage model which describes the influence of age, time and duration of exposure. This is not always a straightforward process, and in some situations significant errors have been made in formulating the scientific basis for a particular risk assessment.

(4) The limited experience available from human studies suggests that, in most cases, the exponent of dose in the dose–response function for carcinogens is not less than unity.

(5) Ideally, chemicals which are hazardous to health would be identified before appreciable human exposure has occurred, and then excluded from the

human environment. The early identification of chemicals which produce late adverse effects depends, in most cases, on the results of studies in experimental animals. The use of experimental studies for this purpose could be greatly improved by an adequate comparison of the effects in humans, animals and other biological systems of chemicals for which human response data are available.

(6) Risk analysis and projection of carcinogenicity data may be aided by the use of carefully selected mathematical models which describe the relationship between incidence and dose. The choice of model used for quantitative risk analysis has a profound influence on the magnitude of risk estimated, particularly at low doses. The fact that models are compatible with current knowledge of underlying mechanisms and fit data obtained in animal and human studies, does not prove that the underlying assumptions or the extrapolation are correct.

(7) Risk estimates derived by using the linear non-threshold extrapolation model are commonly regarded as plausible 'upper limit' estimates for the species under consideration. It is not likely that the true risks would be much higher than the risks estimated with a linear model, and it is possible that they could be considerably lower. Having established the upper limit of risk, interspecies differences in susceptibility should be taken into account. However, for most chemicals, data on this important variable are not available. Given these constraints, upper-limit risk estimates may be useful.

3.2.6 Recommendations

(1) Development of regulations for, or legislation on, the collection of quantitative data on occupational exposures to chemicals for which there is sufficient evidence of carcinogenicity or mutagenicity, with corresponding records on those exposed, should be given a high priority.

(2) International guidelines for standard procedures of measurement and reporting of carcinogens or mutagens in the workplace and the general environment should be established to facilitate amalgamation of data from different studies.

(3) Efficient access to essential data, especially of individual records of occupational exposure to known or suspected carcinogens and mutagens and to individual death records, should be guaranteed by law subject to appropriate methods of privacy protection.

(4) The feasibility and utility should be examined of collection and storage of biological and environmental samples from industrial situations, for subsequent exposure estimation in case-control or follow-up studies and for other purposes.

(5) A repository of information about collections of biological material from identified individuals or environmental samples from identified exposure situations, should be established so that it could be used now for the purposes listed in (4) above.

(6) Where possible, data should be collected at autopsy on the distribution by organs of carcinogenic or mutagenic substances in exposed individuals.

(7) *In vivo* methods of measurement of past and present exposure to carcinogens and mutagens and early indicators of their action should be developed and used. Epidemiological studies of effects such as benign lesions and chromosome breakage may give early and sensitive warning of more serious consequences of exposure.

(8) International registries of highly exposed groups should be established to facilitate subsequent follow-up of the incidence of cancer and other late effects of exposure (for example, persons involved in industrial accidents with exposure to chlorinated dibenzodioxins, persons with accidental heavy exposure to PCBs, persons involved in the manufacture of phenoxyacetic acid herbicides and chlorophenols).

(9) Exposure to chemical carcinogens in medicines, with specific dose information, should be registered for subsequent linkage to data from cancer surveillance systems.

(10) Existing banks of data on individuals, such as the US National Health Examination Surveys, should be exploited more extensively through linkage with cancer registries, death registries and other disease surveillance systems.

(11) Multiple endpoints, such as indicators of somatic cell damage, germ cell effects, evidence of carcinogenesis and other biological effects, should be considered for inclusion in any large-scale epidemiological study so that the most efficient use is made of the available research resources.

(12) In the absence of biological evidence favouring a contrary approach, non-threshold linear extrapolation of human dose–response curves should be used for pragmatic estimates of cancer risk at low doses of carcinogens. In most instances, this approach is likely to provide a plausible upper bound to the expected risk.

(13) Provision should be made for independent review of the scientific basis or risk assessments prepared for regulatory agencies.

(14) More data on known human carcinogens from experimental systems *in vivo* and *in vitro* should be collected to permit quantitative cross-species comparisons of carcinogenic and other relevant responses.

(15) A focused programme of experimental studies (bioassays) of carcinogens, for which quantitative dose–time–response data are available in humans, should be initiated so that the corresponding quantitative response data become available from *in vivo* and *in vitro* experimental systems.

(16) The mathematical model selected for quantitative risk estimation should be compatible with the underlying biological mechanism, and should be demonstrated to agree with appropriate data on animals and man.

(17) A coordinated programme of epidemiological studies is required for chemicals for which there is substantial evidence of carcinogenicity or mutagenicity in experimental systems and to which human exposure has occurred but for which human response data are lacking.

3.3 RISK ESTIMATION FOR OTHER TOXICOLOGICAL ENDPOINTS*

In contrast to the previous parts of the Joint Report, this section deals with methods for estimating risk for toxicological endpoints (effects) other than cancer and mutations. Thus, its scope includes reversible and irreversible toxicity to all organs and body functions resulting from short-term, medium-term (3 months) and long-term exposures.

Exposures to chemicals are known to produce a diversity of biological effects which may influence physiological functions such as reproduction, growth, respiration, absorption and assimilation of nutrients, excretion of waste products, muscular activity, nerve transmission, immune response and the overall homoeostatic control mechanism of the body. Subtle effects of certain chemicals on behaviour have also caused concern. The type and intensity of adverse effects depend on the toxicity of the chemical and on the level and duration of exposure.

The toxicological data base for most chemicals in use today is built on information derived almost exclusively from animal experiments. The first step in risk assessment is to make the general assumption that if a chemical produces a given adverse biological effect in one or more species of test animals, it should be treated as potentially toxic to man unless proven otherwise. Unlike pharmaceuticals, for which regulatory procedures permit limited clinical trials before their release to the market, other chemicals are not subjected to any clinical evaluation before marketing. Information on direct health effects in man is derived either from reports of cases of acute poisoning or from case studies of persons occupationally exposed. Epidemiological techniques have been increasingly used in recent years and have generated useful data.

Risk/benefit assessments have been in some cases performed without either quantitative or quantifiable information on risks involved (for example, the use of organochlorine pesticides in the control of vector-borne diseases).

Initial studies in laboratory animals should be conducted using a dose–response design in order to permit at least a semiquantitative selection of potentially 'critical' adverse effects. In general, the adverse effects produced at the lowest dose tested and having the greatest likelihood of public health impact should be further investigated to identify the 'critical' effects possibly by using methods for quantitative estimation.

3.3.1 Current Practice

The usual approach to setting exposure limits for biological effects other than cancer has been the identification of the 'no-effect-level' (NOEL) (or more

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correctly 'no-observed-effect-level') and the application of some safety factor to obtain the acceptable daily intake (ADI) for the chemical considered. National and international organizations have most often used a safety factor of 100 which incorporated a 10-fold margin for interspecies variability and a 10-fold margin for variability among human individuals. However, margins of safety used have been as small as 2 to accommodate either appropriate therapeutic ratios or engineering feasibility; and they have been as large or larger than 5000 to express concern about inadequate data and/or the severity of the effect (WHO, 1978; NRC, 1980).

The use of NOEL and safety factors has several drawbacks:

(1) it uses the lowest and weakest point in the test and disregards the remainder of the dose–response curve;
(2) it assumes that the threshold in a small group of laboratory animals is identical to the threshold for a much larger population of human subjects; and
(3) it uses safety factors that are to a large extent arbitrary.

Other more scientific procedures should be developed and compared to this method.

Terms such as risk, hazard, safety and threshold have widely different meanings to different persons, thus enhancing the possibility of misunderstandings. Therefore, for the purposes of this section of the Joint Report, definitions proposed in a document issued by the World Health Organization have been adopted (WHO, 1978). It is important to emphasize that risk, hazard and threshold are scientifically definable terms, whereas safety connotes the social acceptability or toleration of risk.

3.3.2 Approaches to Quantitative Risk Estimation

The objective of this section of the report is to explore the feasibility of applying quantitative risk estimation concepts and methods to effects other than cancer and, if possible, to describe general procedures by which such risk estimates could be made.

Two broad classes of data are generally available for estimating risks. The first class includes human clinical or epidemiological data which are generally supported by data obtained with laboratory animals. The second class contains only the results of laboratory animal studies.

Ideally, evidence should be obtained of the effects of chemicals in human subjects including the low-dose range. However, such information is rarely available, both for technical and ethical reasons.

The results from human studies may require quantitative extrapolation from observed exposures (for example, occupational) to lower exposures in the general

environment, whereas interspecies extrapolation or transposition is not necessary. Laboratory animal and other studies—including those using appropriate *in vitro* models—can provide useful information for the elucidation of pathogenesis and for the identification of factors that may modify the expression of injury.

More frequently only animal data are available for the estimation of risk. Two extrapolation steps are then required:

(1) extrapolation from high to low doses and
(2) quantitative extrapolation (transposition) from the species tested to man.

In principle, methods used for extrapolating experimental animal cancer data from high to low doses can be applied to non-cancer endpoints. However, because of different mechanisms of action of carcinogens and other chemicals, mathematical expressions different from those used for carcinogenicity may be needed for the quantitative extrapolation of other toxicological data. Also, many non-cancer effects are believed to have a threshold, and carcinogenesis is usually considered as a non-threshold phenomenon. Although the dose–response relationships for carcinogens and non-carcinogens can be different, it is important to recognize that population thresholds for an exposed human population can be only seldom, if ever, defined. It has been often assumed that the thresholds obtained in laboratory animal studies are directly applicable to human populations. True population thresholds may or may not exist, but the extrapolation of observed threshold levels from one species to another is not appropriate because of the differences in sensitivity and genetic heterogeneity between laboratory animals and human populations, and because of practical limitations of the 'true' threshold detection inherent in studies with laboratory animals. The threshold concept has been reviewed (WHO, 1978), and the reader is referred to that publication for additional details.

Low-dose extrapolation is based on mathematical models which either predict the response at a given low dose or the dose for a predetermined low response. For details on the models used for carcinogens, the reader is referred to the section on epidemiology, statistics and mathematical modelling, to the paper by Hoel (this volume) and to the WHO (1978) monograph. Models for the estimation of critical concentrations for the toxicity of some metals and organometallic compounds have been developed and applied (Nordberg, 1976). The probit and Weibull models have been applied for the description and extension of dose–response curves for non-carcinogenic effects of several compounds (Biddle, 1978; Finney, 1978; NRC, 1980). Another approach applicable both to chemical carcinogenesis and other toxicological effects is the linear interpolation method (Gaylor and Kodell, 1980).

A valid approach for estimating low-dose risk—and one that would avoid interspecies extrapolation—would be to use epidemiological data showing an exposure–response relationship, combined with an adequate understanding of

human tissue sensitivity and of human metabolism of the chemical under consideration. Such data, when available, provide a sound basis for low-dose extrapolation.

Either of these two approaches may alone provide an adequate basis for the estimation of low-dose risk. The use and limitation of data on human toxicokinetics and metabolism and on tissue sensitivity (i.e. toxicodynamics) are less familiar. The application of this approach leads to an expression for a complete dose–response curve (see Nordberg and Strangert, this volume), including kinetics of uptake, distribution, biotransformation and excretion. Such data, including those on tissue sensitivity, however, are rarely available for human subjects (see Bass *et al.*, this volume).

When human data are insufficient for the application of the approaches outlined above, they may be supplemented with animal data. For example, in the 'metabolic modelling approach' (see Nordberg and Strangert, this volume) human data from a few case reports are combined with whole animal and *in vitro* data to predict human dose–response patterns. In spite of introducing the uncertainty of extrapolating from animal to human tissues, this procedure is likely to be more certain than the more direct extrapolation from whole animals to humans. This method has already been applied and proved useful in the analysis of chemical embryotoxic agents (see Bass *et al.*, this volume).

The proper application of these methods of quantitative risk estimation requires some understanding of the mechanisms of action. Additionally, detailed studies on the mode of action of a chemical may eventually allow some generalizations regarding the dose–response relationships that apply to particular categories of chemicals and effects. This has already been discussed for carcinogenesis. However, for most biological effects of chemicals, these mechanisms of action are not well understood. An example is fertility, which is only one of many possible toxicological endpoints in the reproductive process. Fertility is the final expression of a complicated system of functions and may be impaired by chemicals which, for instance, interrupt a crucial stage of spermatogenesis, reduce libido or deplete the number of oocytes (see Wilcox, this volume). In each case, a distinct process of chemical action and host reaction has to be expressed as a specific dose–response relationship. This variety of necessary investigations represents a fundamental challenge to researchers on the estimation of risk of adverse effects other than carcinogenesis.

When estimating the risk on the basis of either human data or laboratory animal data, pathological changes whose severity and importance to health makes them particularly critical should be identified. Although experience has shown that only one effect is usually the most critical for human health and the risk estimation has to be performed with regard to this effect, it is advisable to make risk estimates for all serious pathophysiological alterations. This permits the decision maker to rank the effects by societary importance and to apply different risk tolerances to different adverse effects. It may be useful to rank risk

estimates for each chemical in order to obtain an indication of potential health impact.

3.3.3 Presentation of Risk Estimates

In order to reduce uncertainties about the accuracy of risk estimates and to minimize the underestimation of risks, it has been suggested that the results of quantitative risk estimation should be expressed as an upper bound of risk (for example, as the upper 95 % confidence limit). However, the selection of either the upper confidence limit or the best point estimate of risk is a policy decision that is influenced by the degree of conservatism acceptable and the level of confidence to be achieved. When risk estimates are being used to choose the safer of the two alternatives, the comparison should be made between the best available point estimates and not between the upper bounds of risk estimates for each alternative. The use of the upper confidence limit may cause problems when comparing two risks.

In the present state of knowledge, a quantitative risk estimate should never be expressed only as a single numerical value; it should include a narrative and, if possible, numerical confidence limits. The narrative must express the limitations of the data on which the estimate is based and the implicit and explicit assumptions which have been made (for example, the existence or absence of information on metabolism and on species variability). There is practically no experience today to assess the validity of quantitative risk estimates based solely on experimental animal data. It must also be emphasized that the numerical estimates may be modified by a variety of host and environmental factors (for example, hormonal status, intercurrent disease, age, diet, smoking and occupational setting).

3.3.4 Conclusions

(1) As regards data on which risk estimations are based, there are two main approaches:

(a) primary reliance on human data with supportive information from laboratory animal studies; and
(b) primary reliance on data from laboratory animal studies with or without human data.

Confidence in quantitative risk estimates is likely to be higher in the first approach than in the second. When using human data, the major difficulties are the extrapolation from high to low exposures and the adequate collection and proper use of exposure data; whereas for the second approach the major difficulties include not only extrapolation from high to low doses but also extrapolation (transposition) across species.

(2) When using laboratory animal data on toxicity to obtain acceptable exposure limits for effects other than cancer, the application of safety factors has been the most common procedure, but safety factors are arbitrary and include a component of social and policy judgment. New and better methods have not been sufficiently explored or agreed upon. Therefore, the use of NOEL and safety factors cannot be completely replaced at the present time.

(3) Mathematical methods for quantitative risk estimation have been applied effectively to the estimation of risk associated with exposures to metals and metal compounds and to other substances such as pharmaceuticals. Some of these models are more complex than the conventional curve fitting and do take into account biological processes such as metabolism and toxicokinetics. It is recognized that quantitative estimation of risk in humans based solely on laboratory animal studies involves a substantial uncertainty. Such estimation is not reliable until better biological models become available.

(4) Because non-cancer effects are known to involve a wide variety of mechanisms, it is likely that different mathematical models may be needed. Because the mechanisms of chemical carcinogenesis may be quite different from the mechanisms of non-carcinogenic toxic action, the direct application of mathematical models used in cancer studies does not appear feasible. Quantification of risks associated with exposure to chemicals could improve the understanding of the contribution of chemical exposures not only to the incidence of cancer but also to the incidence of some other non-communicable human diseases with multifactorial pathogenesis.

3.3.5 Recommendations

(1) Approaches, additional or alternative, to the use of NOEL and safety factors should be explored to possibly enhance the scientific credibility of risk estimation in humans.

(2) Improvements should be sought in the toxicological (including toxicokinetic) methods for low-dose extrapolation within the same species with emphasis on chronic low-level exposure.

(3) Biological indices of human exposure to chemicals should be further explored.

(4) The ways in which epidemiological data are collected should be modified to improve dose–response information obtained from human studies.

(5) Novel approaches and methods should be developed to improve quantified interspecies extrapolation.

(6) A better understanding of biological mechanisms is required as a basis of mathematical models for risk estimation.

(7) Greater attention should be paid to the understanding of non-carcinogenic toxicity of chemicals.

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