1 Introduction, General Conclusions, and Recommendations

1.1 INTRODUCTION

Numerous chemical and physical agents in the environment are capable of reacting with DNA. These potential DNA-damaging substances include not only man-made compounds, but also those of natural origin (e.g., food constituents and ultraviolet radiation). Fortunately, mammalian species possess powerful defence systems to overcome potentially harmful effects of dangerous agents. These systems comprise: (a) metabolic and pharmacokinetic processes that determine the absorption, movement, alteration, and detoxification of xenobiotic chemicals within organs and tissues of the body; (b) cellular DNA repair mechanisms; and (c) immunological and other defence processes for diseases such as cancer.

The complex nature of both the intrinsic and acquired potential for DNA damage from a myriad of chemical and physical agents together with limited knowledge of the organotropic and cellular defence mechanisms makes risk assessment, both qualitatively and quantitatively, of adverse biological responses in exposed humans very difficult. Consequently, due to developmental and economic factors, only limited test systems can be applied in practice. Moreover, tests in humans and even in some experimental animals must be restricted and, therefore, considerably limit confidence in extrapolation of results.

This monograph and the workshop from which it is derived are an attempt to evaluate the current state of knowledge of defence mechanisms and to apply this knowledge to estimate risk to health of humans exposed to substances that alter genetic material. The analysis is meant to be an overview rather than an exhaustive treatment of new and promising approaches. To limit possible bias introduced by a "windowed" view from the use of a particular species, a specific endpoint, or a limited class of DNA-damaging agents, emphasis is placed on integrating information obtained from various disciplines.

This evaluation deals predominantly with mechanisms of activation and detoxification of DNA-damaging agents, organotropic and cell-structure effects, the induction and repair of DNA damage, and the molecular and phenotypical analysis

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of mutation induction. Because DNA damage is the initiation step of diseases of genetic origin, this report highlights cancer as the adverse biological response of primary concern; therefore, the processes influencing promotion and progression of transformed cells are not included. For mechanistic studies relied upon in the current practice of risk assessment, several approaches are considered: (a) DNA adduct formation is viewed as a "key event" in the process of cancer formation; (b) the identification of agent-specific "key mechanisms" involved in the initiation of cancer; and (c) application of experimental tests applied among species to rank carcinogenic potency of chemical carcinogens—but only when the nature of such "key mechanisms" is known (e.g., the relative reactivity of various monoalkylating agents toward different nucleophilic centers in DNA, or the role of recombination events in the working mechanism of bifunctional agents). Two other reviews of this subject are recommended to readers: National Research Council (1989) and Volume **98** of *Environmental Health Perspectives* (1991).

In many cases, however, agent-specific "key mechanisms" are unknown. Risk assessment, in practice, is based on the evaluation of test results from a battery or from tier systems, or through interpretation of the total activity profiles of genetic and related effects (Genetic Activity Profiles [GAP], Waters *et al.*, 1987). Ultimately, ranking chemicals through analysis of the GAPs can be achieved through a consensus approach of ICPEMC (Lohman *et al.*, 1990); however, this ranking will not necessarily reflect the carcinogenic potency of chemicals involved in the study (Waters *et al.*, Chapter 13).

Genotoxicity can be compared qualitatively and quantitatively with the structure of compounds. This approach is called "structure–activity analysis" (SAR), whose aim is to differentiate molecular fragments within chemicals that impart a greater or lesser probability of biological injury for humans. However, limitations in our knowledge greatly restrict SAR to a few biological endpoints.

1.2 CARCINOGENESIS AS A MULTISTEP PROCESS

Carcinogenesis, a complex multistep process, may vary with the type of carcinogen, target site for tumour induction, and species. Although the actual mechanisms responsible for the induction of cancer are unknown, two or more genetic events may be involved, and cell proliferation is required. Genotoxicity can result from point mutations, chromosomal rearrangements, recombination, insertions or deletions of genes, and gene amplification. Cell proliferation is required to convert DNA damage into mutations and for clonal expansion of initiated cells, which also increases the probability of additional genetic events occurring in initiated cell populations.

For the majority of human carcinogens and for many carcinogens in laboratory animals, DNA damage is produced by electrophilic attack by the parent compounds or via their metabolites—hence, use of the term "genotoxic carcinogen." For the remaining carcinogens, their mechanism(s) of action appears not to involve detectable DNA damage—thus, they are referred to as "non-genotoxic carcinogens"

or "tumor promoters."

Major steps in the multistage process of chemical carcinogenesis are summarized as follows. Chemical carcinogens to which exposure takes place through ingestion, inhalation, or dermal contact are absorbed, distributed, metabolized, and excreted with rates that are determined by the chemical properties of the carcinogen and by biochemical and physiological factors of the host. Many biotransformation pathways produce detoxified products that are excreted. Chemically reactive electrophilic derivatives produced through similar metabolic pathways react with available nucleophilic centers in cellular constituents, including DNA, RNA, and proteins. Formation of covalent adducts with nucleophilic sites in DNA is vital to induce mutations which, according to prevailing opinion, are the initiating events in carcinogenesis. Most carcinogens produce a complex spectrum of adducts through electrophilic attack on multiple nucleophilic sites on DNA bases. These adducts vary in structure, stability, and, as a consequence, ability to induce mutations. Both quantitative and qualitative features of the adduct profile for any given carcinogen are known to be determinants of the carcinogenic response. The number of adducts formed in cellular DNA of humans or animals exposed to carcinogens is influenced by: (a) kinetics of absorption, distribution, and excretion of the parent substance; (b) enzymatic competence for metabolic conversion to electrophilic derivatives; and (c) cellular content of protective factors such as glutathione. Each factor is important in the determination of organotropic, interspecies and intraspecies differences in susceptibility.

DNA repair processes also affect both quantitative and qualitative characteristics of carcinogen–DNA adduct profiles. Mechanisms of DNA repair have been characterized extensively in prokaryotic cells. Although the process in higher organisms appears to share some features in common with that in prokaryotes, the process appears more complex in eukaryotic cells. Processes have evolved to more effectively repair damage to functionally important lesions (i.e., in transcriptionally active genes) than untranscribed regions. Several types of alterations in gene structure (namely, point mutations, deletions, sister chromatid exchanges, and chromosomal aberrations) are created by replication or recombination of damaged DNA. Accumulation of these lesions leads to the initial stages of transformation. Certain characteristics of the DNA repair process, such as the influences of adduct structure or transcriptional activity on repair efficiency, may be important influences on mutagenesis, and, therefore, may alter carcinogenic susceptibility.

1.3 REACTION KINETICS AND ADDUCT MONITORING

Sufficient information exists about the causal relationship between DNA damage induced by genotoxic chemicals and the occurrence of chemically induced tumours for DNA adducts and DNA repair to be recognized as sentinels of exposure to chemical carcinogens. Therefore, the following premise is widely supported: A population whose DNA contains adducts derived from a known chemical

carcinogen is likely to develop a higher incidence of tumours than a matched control group having far fewer adducts. The question thus arises: Can individuals in multiple groups be sorted according to levels of adducts and to levels of cancer risk consistent with levels of adducts? Presently, this separation cannot be accomplished for the following reasons:

1. Covalent binding of chemicals to DNA is an imprecise concept. That is, some DNA adducts are of greater significance with respect to carcinogenic outcomes. Specifically, some types or locations of adducts are removed faster than others, while others go unrepaired. Furthermore, the relative level of various adducts will change temporarily according to alternative rates of repair. In some cases, the *presence* of an adduct leads to mutation via miscoding during replication; whereas, in other cases, the error-prone *removal* of adducts leads to mutations. To complicate matters further, each factor varies according to the chemical, species, strain, sex, or tissue under study.
2. DNA adduction, damage, repair, and mutation represent only the initiation steps in the multistep process of carcinogenesis. Thus, the response of an individual to a given initiatory event will vary according to a wide range of influences referred to as toxicity, promotion, progression, immune response, and individual sensitivity.

Consequently, the measurement of adducts, DNA repair, and mutation provides a list of points of departure for complex phenomena whose outcome is certainly to be decided by a variety of additional factors. The absence of a correlation between adducts in tissues and carcinogenicity in organisms may be inevitable, because not all tissues with adducts develop cancer. Nonetheless, in cases where a compound-specific adduct is identified in a tissue that subsequently develops tumours, then positive correlations have been observed between these two parameters.

The previous considerations can have a perverse effect on seeking simple correlations. For instance, although two methylating agents (e.g., MNU and DMS) yield grossly similar levels of DNA methylation, minor O^6guanine (O^6G) methylation products derived from MNU are the determinants of cancer risk. This source of confusion may be magnified when adducts are measured in DNA surrogates such as protein or haemoglobin, leading to the possibility that the greater the extent of protein adduction, the less adduction at critical sites (e.g., O^2thymine (O^6T) or O^6G). This situation requires considerable caution when going from the qualitative assessment of exposure to carcinogens to extrapolating adduct data as a measure of carcinogenic risk. A fertile area of research to overcome such limitations is perhaps prospective epidemiology which measures secondary variables to seek correlations with incidences of cancer that become available in the future.

1.4 GENERAL RECOMMENDATIONS

1. Tolerance mechanisms for unexcised damage to DNA need to be further characterized, because they may play an important role in mutagenesis. Excision–repair is not the only way in which cells handle DNA damage. Tolerance processes (daughter-strand repair) exist whereby cells are able to cope with unexcised damage during DNA replication.
2. Research is needed into the efficiency, heterogeneity, and fidelity of the excision–repair process in different species, and in different tissues and stages of development within an organism. This effort should include purification of excision–repair gene products. Excision–repair in mammals is a highly complex process involving many gene products. More than seven proteins are required to carry out the incision step which can be effected by three polypeptides in *Esherichia coli*. The individual role of these many gene products in this process and their mechanism of action is not yet understood.
3. Research using whole animals is needed to define the extent to which interspecies similarities and differences can be extrapolated from cultured cells. Studies of molecular characterization of excision–repair in mammals, confined largely to cultured cells, have pinpointed some interspecies differences, e.g., the apparent inefficiency of rodent cells in excising cyclobutane dimers in the bulk DNA, when compared with human cells.
4. Relevant animal models should be developed to understand the influences of other factors on the relationships between repair, mutagenesis, and carcinogenesis. Such models are now becoming feasible as a result of measurements of targeted mutagenesis of repair genes and the production of transgenic mice engineered to be deficient in DNA repair. Such repair-deficient mice having heightened sensitivity to cancer induction could also serve in testing chemicals for carcinogenicity. From the xeroderma pigmentosum paradigm, defective repair has been implicated in increased mutagenesis and carcinogenesis; yet this relationship is quite simplistic. In Cockayne's syndrome and trichothiodystrophy, defects in DNA repair result in pathologies in the apparent absence of cancer.
5. The window through which one looks at mutational events should be broadened, for instance by determining mutation spectra in a variety of reporter genes.
6. Unselectable markers should be utilized to investigate mutation induction in transcriptionally inactive loci which might be relatively poorly repaired. Mutational events in such loci might become important when expressed later in life. Presently, mutation spectra are determined only in selectable marker genes.
7. Spectra in repair proficient and deficient conditions should be compared.
8. Investigations should be undertaken of the precise mechanism of mutation fixation. This objective can be achieved by using mutagen-sensitive mutants which are not deficient in DNA repair or via homology with yeast or *E. coli*.
9. Mutational spectra should be determined in whole animals using intrinsic genes, restricting the analysis mainly to one cell type, e.g., T cells or germ cells.
10. Transgenic organisms should be developed to study organ specificity among species and to facilitate risk assessment. While transgenic plant and fish can be

produced, no reporter gene designed for mutation detection has been incorporated in these organisms.

1.5 REFERENCES

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