Assessment of the Carcinogenicity and Mutagenicity of Chemicals

Report of a
WHO Scientific Group

Technical Report Series 546

World Health Organization, Geneva 1974

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

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Geneva, 13–17 August 1973

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ASSESSMENT OF THE CARCINOGENICITY AND MUTAGENICITY OF CHEMICALS

Report of a WHO Scientific Group

A WHO Scientific Group on the Assessment of the Carcinogenicity and Mutagenicity of Chemicals met in Geneva from 13 to 17 August 1973. The meeting was opened by Dr P. Dorolle, Deputy Director-General, who welcomed the participants on behalf of the Director-General.

1. INTRODUCTION

For a number of years WHO and FAO have convened regular joint meetings of experts on food additives and on pesticide residues. The experts have been given the task of evaluating the toxicity and establishing acceptable daily intakes for food additives, pesticide residues, and contaminants that are important from the point of view of health and the supply of food.

In evaluating the toxicities of these chemicals, various problems have been encountered. One of these concerns the significance of exposure to very low levels of substances that have been shown to be carcinogenic or mutagenic in laboratory investigations. Some of these substances can be eliminated from the food; others cannot be readily eliminated. A realistic assessment must therefore be made of the health hazards, if any, that are involved in such exposures. This problem is not a new one, but recent developments in the relevant disciplines may be helpful in providing an answer.

In addition, the possible mutagenic action of food chemicals poses a definite health hazard. The WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives [1] reached the conclusion that while this problem cannot be ignored there are considerable difficulties in extrapolating from the experimental data to possible hazards of food additives in man. In recent years, however, a number of testing procedures have been developed. These involve the use of mammals, including human cell systems. A Scientific Group on the Evaluation of Testing of Drugs for Mutagenicity [2] was therefore convened by WHO in

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 482.

1971 to discuss, among other items, methods of testing and the interpretation of results. It is recognized, however that some new information has been accumulated in this field in the past two years.

Although the Group recognized that in many instances a critical analysis of benefits versus risks was valuable, discussion was limited to the evaluation of risks.

2. MECHANISMS

2.1 Mutagenesis

It is now accepted that the genetic material of all living organisms, with the exception of some viruses, is DNA. The genetic information is encoded in the sequence of base-pairs such that three bases specify one protein amino acid. The code is said to be universal, meaning that the same three bases (triplets) correspond to the same amino acid in all living systems. The double helical structured DNA proposed by Watson and Crick has made it possible to explain replication of the genetic material and mutations in chemical terms.[1]

A number of chemicals, including alkylating agents, analogues of DNA bases, and other types of molecule have been shown to induce mutations in biological systems, ranging from viruses to mammals. More recently the possible hazard to man from the presence of mutagenic chemicals in the environment has been recognized.

Mutations are classified as gene or point mutations, which may result from changes in one or a few bases, and as microscopically visible changes in the structure or number of chromosomes, which involve changes in many more bases. Point mutations may arise either by base substitution or frame-shift mechanisms. Base substitution can occur by incorporation of base analogues into the DNA, leading to mispairing on subsequent replication or by chemical reaction with a base already present in the DNA chain, giving rise to an abnormal base, which mispairs at the next replication. Frame-shift mutation involves addition or deletion of bases in the DNA and is induced by agents that, because of their size and shape, can become intercalated between the base pairs.

Heritable visible chromosome aberrations may follow exposure of cells to chemical mutagens. There are cellular mechanisms, described later in the report, that can repair lesions in DNA. The importance of DNA repair processes in human disease is illustrated by the rare condition xeroderma pigmentosum, which is genetically determined and in which

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[1] Watson, J. D. (1970) *Molecular biology of the gene, 2nd ed.*, New York, Benjamin, p. 662.

cellular repair of DNA lesions induced by ultraviolet light is defective.[1] Sufferers from this disease have a greatly increased incidence of active skin tumours.

2.2 Carcinogenesis

Many, perhaps most, chemical carcinogens are thought not to be carcinogenic themselves but to require metabolic activation in the body to form active products which induce cancer. This activation is usually mediated by tissue enzymes, which occur mainly, but not exclusively, in the liver. Sometimes, however, activation is mediated by enzymes of the microbial flora of the intestinal tract. The terms precarcinogen, proximate carcinogen and ultimate carcinogen have been introduced by Miller & Miller[2] to describe respectively, the compound administered, its metabolites with increased carcinogenic potency, and the final metabolic product that is thought to react with a cellular component or components to induce the malignant transformation. In spite of the very varied chemical structures of the known precarcinogens there is evidence that many of them are converted in the body to electrophilic reactants, which interact with various nucleophilic centres in the cell, including nucleic acids, proteins, and protein-bound methionine. Similar conclusions apply to the metabolic activation of some mutagenic chemicals.

The need for metabolic activation of some carcinogens and mutagens has important implications for the design of *in vitro* tests for both activities. With some chemicals positive results can only be obtained in the presence of suitable metabolic activating systems.

Although the facts of interaction of the active forms of chemical carcinogens with cellular macromolecules are well established, the significance of these interactions for carcinogenesis is not yet understood. Reaction with DNA gives support for the idea that cancer can result from mutation of a somatic cell and thus for a close interrelation between carcinogenesis and mutagenesis. This is no more than a hypothesis, however, and various epigenetic mechanisms of cancer resulting from interaction with cellular RNA or proteins have been put forward. Recently, there has been much renewed interest in the idea that chemical carcinogens may activate latent tumour viruses already present in the cell. The above considerations probably apply only to those carcinogens that react covalently with cellular

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[1] Cleaver, J. E. (1968) *Nature (Lond.)* **218**, 562.

[2] Miller, E. C. & Miller, J. A. (1971) *The mutagenicity of chemical carcinogens: correlations, problems, and interpretations.* In: Hollaender, A., ed., *Chemical mutagens*, New York, Plenum, Vol. 1, pp. 83–119.

macromolecules. Other types of carcinogenesis that may involve indirect mechanisms are discussed later in this report.

3. RELATIONSHIP OF MUTAGENESIS AND CARCINOGENESIS

Current theories postulate similarities between the mechanisms of mutagenesis and the mode of action of major groups of chemical and physical carcinogens.

There is increasing evidence that many chemical carcinogens in their carcinogenically reactive form can induce mutations in microbial and some mammalian test systems. But it is impossible to assess whether or not these common properties of many chemical carcinogens and mutagens also point to common sequences of events resulting in a cancer cell or a mutated cell. Furthermore, some potent mutagens do not appear to be carcinogenic in any of the test systems used and certain carcinogens have not been demonstrated to be mutagenic. One major difficulty in the comparison of mutagenic and carcinogenic actions is the use of results obtained from different test systems. Induction of point mutations is reported mostly from studies in microbial systems, whereas chromosomal abnormalities have been observed in tissue culture and, more recently, *in vivo*. Carcinogenicity, on the other hand, is reported largely from *in vivo* studies in rodents. A second difficulty arises from the need for metabolic activation of many chemical mutagens and carcinogens. Until recently most *in vitro* systems used in mutagenesis bioassay have lacked this activation potential. It is thought that metabolic activation converting a precarcinogen into the "ultimate" carcinogen is analogous to the change from a premutagen to the ultimate mutagen.

4. COMMENTS ON MUTAGENICITY AND CARCINOGENICITY TESTS

The procedures for testing food additives and drugs and the interpretation of the results have been outlined in the fifth report of the Joint FAO/WHO Expert Committee on Food Additives [1] and the report of a WHO Scientific Group on the Principles for the Testing and Evaluation of Drugs for Carcinogenicity.[2] The *in vitro* test systems using cell transformation

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1961, No. 220; *FAO Nutrition Meetings Report Series*, 1961, No. 29.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 426.

in tissue culture as an end point hold promise as a substitute test for the animal carcinogen bioassay.

Other test systems make use of the capacity of some, if not all, reactive forms of chemical carcinogens to interact with DNA. Mutagenicity tests may have value as a prescreening procedure for carcinogenicity. However, for the time being the development of a tumour, verifiable histologically, in the whole animal must be the ultimate test for carcinogenic activity.

Test systems for mutagenicity of chemicals have been evaluated by two WHO Scientific Groups.[1, 2] They agreed that no single test system can detect and characterize all mutagenic agents. Therefore, the use of several tests is desirable and these should primarily be done in mammals.[2] In addition a number of *in vitro* and submammalian test systems might be used to answer specific questions.

Many known mutagenic agents belong to classes of chemicals that need metabolic activation. Lack of metabolic activation has been one of the principal limitations of studies in *in vitro* and microbial systems. Furthermore, the activation process in submammalian systems, e.g., drosophila, might be different from that in mammals and man. The development of *in vitro* systems, including metabolic activation systems derived from mammals or man, may make possible rapid screening of substances. Data from such systems would be of value for setting priorities for more definitive mammalian testing.[2]

5. THRESHOLD

For most biological effects it is assumed from experience that a threshold and a no-effect level exist. Threshold dose levels in mutagenesis have been questioned on the basis of studies of radiation-induced mutations and because mutations may even result from a change in only one base pair in DNA. For carcinogenesis the existence of a threshold has also been questioned because of:

(1) the self-replicating nature of the cancer cell.

(2) the work of Druckrey and others, which has been interpreted to indicate summation of irreversible effects in carcinogenesis (this has been expressed by Druckrey in the equation $Dt^n = k$,[3] where n is greater than 1).

(3) evidence from experiments on tumour initiation and promotion in skin carcinogenesis indicating lasting change induced by one tumour-initiating event.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 482.

[3] D = dose, t = time.

(4) the fact that cancer can occur in response to chemicals, even after single doses, long after their disappearance from the body.

(5) the possibility that cancer may result from mutation in a somatic cell.

The summation effect described by Druckrey and others is not questioned and his equation characterizing carcinogenic potency may be accepted. Nevertheless, every organism has a limited life span and in this sense there is, for each individual, a real threshold. Cigarette smoking is well known to cause human cancer in a dose-related fashion. The demonstration of a decline in the risk of developing lung cancer in ex-smokers means that these effects are partly reversible. Recent work on the initiation and promotion of tumours, in which application of the promoting agent was delayed for a longer time than in the earlier experiments, suggests that the effect of an initiating event may disappear, but this requires confirmation.

Knowledge of molecular biology has developed rapidly ; and it is now known that there are cellular mechanisms for the repair of DNA. These processes include single-strand and double-strand repair by excision or post-replication mechanisms. Most knowledge of DNA repair has come from investigations of microbial systems, but there are reasons to believe that similar processes occur in mammalian cells.

The repair of DNA usually shows exact fidelity but does not always lead to a perfect copy of the original DNA. Improper repair may result in the death or mutation of cells. Deleterious effects are more common after more severe DNA injury and when there is reduced capacity for repair. Impaired efficiency of repair may be genetically determined, e.g., in human subjects with the repair-deficient type of xeroderma pigmentosum. Several agents are known to interfere with DNA repair in microbial or mammalian cells *in vitro*.

In biological systems with an efficient DNA repair mechanism, the implication of an exposure threshold for point mutations and deletions is very strong. However, it is not established if such mechanisms are effectively present in various types of mammalian cell or if these mechanisms function *in vivo*. If cancer results from such mutations in a somatic cell the above conclusions regarding a threshold may apply to carcinogenesis.

A number of chemically induced tumours possess antigenic properties capable of inducing immunological tumour-associated rejection reactions.[1] The existence of immunological surveillance mechanisms that protect the

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[1] Baldwin, R. W., Glaves, D. & Pimm, M. V. (1971). In : Amos, B., ed., *Progress in immunology — First International Congress in Immunology*, New York & London, Academic Press, pp. 907–920.

host against neoplastic cells has been postulated.[1] This is supported by studies on host immunity to autochthonous tumours in man and others.[2] Immunodeficiency diseases lead to an increased risk of neoplastic disease.

The degree of importance of tumour-limiting responses remains to be analysed qualitatively and quantitatively. The dichotomy of the immune response — with mechanisms that both limit and facilitate neoplastic growth — should be kept in mind.

Further basic studies are needed before a correlation between chemical carcinogenesis and host immunity in man can be established.

From these considerations the existence of a threshold may be envisaged. Nevertheless the difficulties of determining a threshold for a population are great. Therefore, mathematically derived conclusions that it is impossible to demonstrate no-effect levels experimentally cannot be ignored.

6. ASSESSMENT OF HAZARDS

The term "carcinogen" has caused confusion because it applies to agents that are so varied in their quantitative and qualitative characteristics that their control requires many different approaches. However, common usage would seem to necessitate retention of the term. Chemical carcinogens can vary in potency in comparable test systems by a factor as high as 10^7.

Since all chemical carcinogens pose a hazard, human exposure must be reduced to the feasible minimum. With compounds such as aflatoxin that may be active in microgram doses the achievement of this objective may raise formidable practical difficulties.

The action of the majority of carcinogenic compounds is associated with preliminary changes (e.g., hyperplasias, cirrhosis) the role of which is not clear. However there are some chemicals that give rise to neoplasms only after the induction of particular pathological effects. For example, the cancers of the urinary bladder observed in rats treated with Myrj 45 (polyoxyethylene monostearate) are thought to have been caused by the presence of bladder calculi induced by the chemical rather than by its direct action. A no-effect level for chemicals that produce tumours in this way may be established.

The evaluation of the carcinogenic effects of administered hormones must take into account their endogenous occurrence and participation in the regulation of physiological functions. If an intake of a hormone does

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[1] Burnet, F. M. (1964) *Brit. med. Bull.*, **20**, 154-158.

[2] Hellström, K. E. & Hellström, I. (1969) *Adv. Cancer Res.*, **12**, 167–223.

not increase its level beyond the physiological range, then it probably represents a no-effect level. The endocrine status of the test species used should be as close as possible to that of man.

The induction of cancer by some carcinogens is attributable to their physical characteristics. For example, some forms of asbestos are carcinogenic in man and animals. This appears to be related to the physical characteristics of the fibres.

In summary, therefore, it would seem logical that tumour induction be considered as a manifestation of toxicity to be studied as an individual problem in each instance. In some cases, the data available may permit the logical determination of a tolerance level whereas in others, currently the great majority, no such approach is possible.

From a practical standpoint, there is sometimes an irreducible environmental background level of certain cancer inducing compounds, such as aflatoxins and polycyclic aromatic hydrocarbons. The toxicologist must take this into account in his evaluation and recommendations.

7. CONCLUSIONS

(1) *In vitro* mutagenicity tests alone cannot yield definitive results applicable to man. Mammalian test systems are more promising but still require further development and experience.

(2) The relationship between carcinogenesis and mutagenesis requires further investigation. However, the association between mutagenicity and carcinogenicity of many compounds is sufficiently great to justify the use of mutagenicity tests as prescreening procedures for possible carcinogens.

(3) It is recognized that there are certain instances of cancer induction that may be secondary to an initial non-carcinogenic effect of a chemical.

(4) The role of modifying factors, enhancing or inhibiting the effect of carcinogens, must be considered.

(5) Assessment of risk must involve a knowledge of the environmental " background " levels of the chemicals concerned.

(6) Newer knowledge of DNA repair mechanisms and of immunological influences may have a bearing on the evaluation of the effects of low doses of chemical carcinogens.

(7) The possible existence of a threshold to the effects of both chemical carcinogens and mutagens should be envisaged (see section 5).

8. RECOMMENDATIONS

(1) WHO should promote the development of approaches and procedures for assessing the risks of low-level carcinogen exposure by extrapolation from experimental bioassay data.

(2) A knowledge of the environmental levels of carcinogens will be extremely useful in assessing their risks. Consequently WHO should promote more research into methods for the detection of these chemicals and coordinate and support international monitoring of the levels of certain of these chemicals.

(3) In those situations where carcinogens are unavoidable, or where the banning of a substance would impose a hardship or an unrealistic economic burden, the toxicologist must assess the risks associated with different levels of exposure. Proposed approaches for such evaluation include those made by Mantel & Bryan [1] and by Albert & Altshuler.[2] All the proposals suffer from lack of sufficient data to establish their validity and/or from arbitrary assumptions that lead to unrealistic estimates. Friedman (see Annex) has proposed the incorporation of the equivalent of a reference standard to make relative assessments possible. This whole area is of great practical importance and it is suggested that WHO should convene a separate meeting to evaluate this subject.

(4) WHO should encourage further work in the following areas:

(*a*) Basic research into mechanisms of carcinogenesis, including research into DNA repair, so that current empirical approaches may be replaced by one with a sound scientific basis.

(*b*) Studies on the effects of intake of compounds having hormone-like actions to elucidate the interrelationship of physiological and pathological effects that may have a bearing on the assessment of toxicity.

(*c*) Pathological examinations in studies of carcinogenesis where the mechanisms may involve possible secondary factors.

(*d*) Research into the design of a practicable test for point mutations in mammalian systems.

(*e*) Additional research concerned with the association of mutagenicity and carcinogenicity.

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[1] Mantel, N. & Bryan, W. R. (1961) "Safety" testing of carcinogenic agents. *J. nat. Cancer Inst.*, **27**, 455–470.

[2] Albert R. E. & Altshuler, B. (1972) *Considerations relating to the formulation of limits for unavoidable population exposures to environmental carcinogens.* In: *Proceedings of the Twelfth Hanford Biology Symposium on Radionuclide Carcinogenesis.*