General Criteria for Assessing the Evidence for Carcinogenicity of Chemical Substances: Report of the Subcommittee on Environmental Carcinogenesis, National Cancer Advisory Board[1,2]

A. INTRODUCTION

The National Cancer Institute of the United States is frequently asked to advise on the possible carcinogenic hazards of substances that might be introduced into, or already exist in, the human environment. On September 19, 1975, National Cancer Program Director Dr. Frank Rauscher, therefore, asked the National Cancer Advisory Board Subcommittee on Environmental Carcinogenesis to develop general criteria for use in the assessment of whether specific environmental agents constitute a carcinogenic hazard in humans.[3] This document represents this Subcommittee's current formulation of these criteria. The criteria adopted are based upon those generally used in the application of the scientific method to any question. In assembling these criteria, the Subcommittee recognizes that at present there is no simple and universal definition of either carcinogenesis or neoplasia. The criteria which are described are general guidelines and no rigid, universal criteria. The complexity of the problem dictates that the evaluation of the potential human hazards of a given agent must be individualized in terms of the chemical and metabolic aspects of that agent, its intended use(s), the data available at the time that the decision must be made, and other factors pertinent to the case under consideration. Each case must be considered on its own and the criteria appropriate for one agent may not necessarily apply to another.

For purposes of clarity, the general criteria have been classified into three groups in terms of the sources of the data: 1) criteria from human studies; 2) criteria from animal bioassays; 3) criteria from in vitro or short-term tests. This does not imply that human carcinogens are distinct from animal carcinogens. Nor does it imply that carcinogens can be identified absolutely by any of the currently available in vitro or short-term tests. Since the extrapolation of data from experimental animal systems to the human is a problem separate from that of establishing the validity of the experimental animal data, the problem of extrapolation is dealt with separately (*see* Section E) in this document.

A major source of data on carcinogenicity comes from bioassays in experimental animals. Experience has indicated that, with one or two possible exceptions, compounds that are carcinogenic in humans are also carcinogenic in one or more experimental animal bioassay systems. In addition, several compounds first detected as carcinogens in experimental animals were later found to cause human cancer. Demonstration that a compound is carcinogenic in animals should, therefore, be considered evidence that it is likely to be carcinogenic in humans, unless there is strong evidence in humans to the contrary.

In this document the term "carcinogen" is used in its broad sense, because in most of the current human epidemiologic approaches and certain animal bioassays it is not possible to differentiate clearly between initiating agents, promoting agents, and certain modifying factors. Any factor or combination of factors which increases the risk of cancer in humans is of concern regardless of its mechanism of action. The criteria listed here apply only to chemical agents. Criteria for the induction of neoplasms by physical agents or by the direct action of viruses have not been considered in this report.

This Subcommittee has found it useful to state generalized definitions of malignant and benign neoplasms, recognizing that in practice the diagnosis of a particular neoplasm is an operational one based on convention, experience, and experimental data.

A malignant neoplasm is composed of a population of cells displaying progressive growth and varying degrees of autonomy and cellular atypia. It displays, or it has the capacity for, invasion of normal tissues, metastases, and causing death to the host. Benign neoplasms are a less autonomous population of cells, exhibit little or no cellular atypia or invasion of normal tissues, and do not metastasize. In particular cases, however, benign neoplasms may endanger the life of the host by a variety of mechanisms, including hemorrhage, encroachment on a vital organ, or unregulated hormone production. The cytologic and histologic criteria utilized in determining whether a lesion is benign or malignant differ depending upon the tissue in which the neoplasm arises. Evaluation of whether a specific lesion is benign or malignant should, therefore, follow standard criteria used by experimental oncologists and pathologists with the emphasis on correlation of the histopathologic pattern with the biologic behavior of the lesion or type of lesion. In equivocal cases, the diagnosis of a specific lesion may require a panel of experts, recognizing that they may not always agree.

Depending upon the particular case, benign neoplasms may represent a stage in the evolution of a malignant neoplasm and in other cases they may be "end points" which do not readily undergo transition to malignant neoplasms. (For more detailed discussions on the definition of benign and malignant neoplasms and their relationship to each other, the reader is referred to references given in the Appendix.)

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[1] *Subcommittee Members*: Dr. Philippe Shubik, Chairman; Dr. William O. Baker, Dr. Edward J. Burger, Jr., Dr. Irving M. London, and Dr. William E. Powers. *Consultants*: Dr. Arnold L. Brown, Dr. Hans L. Falk, Mr. Arthur McGee, Dr. Norton Nelson, Dr. Irving J. Selikoff, Dr. Michael B. Shimkin, Dr. I. Bernard Weinstein, Dr. Gerald N. Wogan, Dr. Ernst Wynder, Dr. Roy Albert, Dr. Herbert Blumenthal, Dr. David B. Clayson, Mr. Jerome Cornfield, Dr. Peter N. Magee, Dr. Henry C. Pitot III, Dr. David Rall, and Dr. Takeshi Hiravama.

[2] *Address reprint requests to* Office of Cancer Communications, National Institutes of Health, Building 31, Room 10A17, Bethesda, Md. 20014.

[3] *See* letter from Dr. Rauscher in "Appendix."

The general criteria listed in this document reflect the judgment of this Subcommittee based on its assessment of the current "state of the art." These general criteria should be reviewed on a continuing basis and revised as necessary in the light of new knowledge. (For more detailed discussions of carcinogenicity and carcinogen assessment, the reader is referred to specific references on this subject in the Appendix.)

B. CRITERIA IN HUMAN STUDIES

An agent—which may comprise a combination of chemicals—is carcinogenic in man if it increases the incidence of malignant neoplasms (or a combination of benign and malignant neoplasms) in humans to levels that are significantly higher than those in a comparable group not exposed (or exposed at a lower dose) to the same agent. If all of the induced neoplasms are benign rather than malignant, then, for the reasons given elsewhere in this document, the agent must be considered a possible carcinogen and it should, therefore, be very carefully evaluated as a health hazard.

Types of evidence suggesting that an agent is carcinogenic in humans include: neoplastic response directly related to exposure (both duration and dose); incidence and mortality differences related to occupational exposure; incidence and mortality differences between geographic regions related to different exposures rather than genetic differences and/or altered incidence in migrant populations; time trends in incidence or mortality related to either the introduction or removal of a specific agent from the environment; case-control studies; and the results of retrospective-prospective and prospective studies of the consequences of human exposure. Clinical case reports may also provide early warning of a potential carcinogen. Negative epidemiologic data may not establish the safety of suspected materials. Negative data on a given agent obtained from extensive epidemiologic studies of sufficient duration are useful for indicating upper limits for the rate at which a specific type of exposure to that agent could affect the incidence and/or mortality of specific human cancers.

C. CRITERIA IN EXPERIMENTAL ANIMAL STUDIES

The carcinogenicity of a substance is established when the administration to groups of animals in adequately designed and conducted experiments results in increases in the incidence of one or more types of malignant neoplasms (or a combination of benign and malignant neoplasms) in the treated groups as compared to control groups maintained under identical conditions but not given the test compound. The increased incidence of neoplasms in one or more of the experimental groups should be evaluated statistically for significance, and the only major experimental variable between the control and the experimental group should be the absence or presence of the single test agent. Such increases may be regarded with greater confidence if positive results are observed in more than one group of animals or in different laboratories. The demonstration that the occurrence of neoplasms follows a dose-dependent relationship provides additional evidence of a positive result.

The occurrence of benign neoplasms raises the strong possibility that the agent in question is also carcinogenic since compounds that induce benign neoplasms frequently induce malignant neoplasms. In addition, benign neoplasms may be an early stage in a multi-step carcinogenic process and they may progress to malignant neoplasms; also, benign neoplasms may themselves jeopardize the health and life of the host. For these reasons, if a substance is found to induce benign neoplasms in experimental animals it should be considered a potential human health hazard which requires further evaluation. In experiments where the increased incidence of malignant neoplasms in the treated group is of questionable significance, a parallel increase in incidence of benign tumors in the same tissue adds weight to the evidence for carcinogenicity of the test substance.

Certain methods (listed below) are important pointers to potential carcinogenicity and cannot be ignored; however, they may require additional studies before extrapolation to particular conditions of human usage can be made. Examples of these methods are:

Bioassays employing inbred strains of animals which develop high incidences of particular tumors in the untreated state. In some of these studies the particular characteristics of the animals and the results obtained may require additional evaluation—in other instances, such well controlled test systems may be quite satisfactory for the establishment of carcinogenicity of an agent.

Bioassays in which, in addition to the test agent, animals are treated with a known carcinogen, or some other foreign material which itself may be carcinogenic or co-carcinogenic.

Bioassays in which the test animals are subjected to grossly unphysiologic and inappropriate conditions, in addition to the administration of the test compound, and there is reason to believe that these unphysiologic conditions may in themselves enhance tumor induction.

Bioassays in which the test compound is given by unusual routes of administration (such as bladder implantation) and there is reason to believe that the tumors that occurred may not be due to a specific effect of the test compound. This does not mean, however, that substances should only be tested in animals by the same route of administration as pertains to human exposure.

Statistically significant positive results in the above types of bioassay do, however, raise the possibility that the test substance may be carcinogenic.

In the evaluation of carcinogenesis data it is important to consider the composition and identity of the chemical substances tested and their stability under conditions of storage and administration.

D. SHORT-TERM OR IN VITRO TESTS FOR CARCINOGENS

A major practical limitation in the bioassay of potential carcinogens is the large number of test animals and the long duration required to obtain results. A number of short-term or in vitro tests are currently under development and appear promising. These include assays for: the induction of DNA damage and repair; mutagenesis in bacteria, yeast, *Drosophila melanogaster*, or in

mammalian somatic cell cultures; and neoplastic transformation of mammalian cells in culture. Other assays that have been employed include the dominant lethal test and studies of chromosomal damage. The latter two tests suffer from the fact that they are frequently nonspecific and/or difficult to quantify. Of the various short-term tests, the Ames *Salmonella* mutagenesis system has been studied the most extensively. Tests based on other in vitro approaches are also being developed.

The intelligent application and interpretation of the in vitro tests must also take into account species variations in factors related to the pharmacologic distribution and metabolism of the parent compound as well as possible species differences in macromolecular repair and host defense mechanisms. A number of approaches addressed to the metabolic aspects are now available, including "host-mediated" mutagenesis assays; the assay of urine and other biologic fluids taken from animals or humans receiving the test compound; the addition of microsomal enzymes and co-factors to the assay system; and the inclusion of specific cells in the assay.

At present, one of the short-term tests can be used to establish whether a compound will or will not be carcinogenic in humans or experimental animals. Positive results obtained in these systems suggest extensive testing of the agent in long-term animal bioassays, particularly if there are other reasons for testing. Negative results in a short-term test, however, do not establish the safety of the agent.

This Subcommittee is enthusiastic about the possible future use of in vitro tests as part of a screening system for potential carcinogens and believes that their further development and validation deserve high priority.

E. EXTRAPOLATION FROM EXPERIMENTAL DATA AND EVALUATION OF HUMAN RISKS

The criteria listed above provide a guide to determining whether a compound is carcinogenic under a specific set of exposure conditions in a given species or subpopulation. Quantitative extrapolation from animal studies for the purposes of evaluating human risks entails large uncertainties at the present time. Each case must be individually evaluated, taking into consideration such factors as adequacy of experimental design, statistical significance of the data, dose-response relations, duration of exposure, route of administration, metabolism (including species variations), host susceptibility, co-factors and other modifying factors, and the amount of the material to which humans will be exposed. The criteria for extrapolation may vary depending on the agent in question. For example, demonstration that a compound only produces subcutaneous sarcomas in rodents may be relevant to a drug that will be injected subcutaneously in humans, but such animal data may not necessarily be appropriate in assessing the risks associated with a substance that humans will only receive orally. Because of the limitations inherent in animal bioassays, a negative result obtained in a particular animal bioassay does not exclude the potential carcinogenicity of a compound in humans. The inappropriate experimental species may have been chosen; the number of animals tested may have been too small; or the duration of observation may have been too short. Alternatively, test conditions may have been inappropriate in terms of their predictive value for the response of humans. The extrapolation of experimental carcinogenicity data to the human situation is strengthened by obtaining results in more than one species. Negative results obtained in one species do not, however, detract from the significance of clearly positive results obtained in another species.

For more detailed discussions of the problems of extrapolation and the estimation of safe limits, the reader is referred to references listed in the Appendix.

F. EVALUATION OF BENEFITS VERSUS RISK

In those cases in which a compound has been proved to be carcinogenic remains the decision to what extent the possible risks to man are counterbalanced by the possible social, economic, or medical benefits of that substance. Scientists must play a major role in these decisions by providing and interpreting the available data. The final decision, however, must be made by society at large through informed governmental regulatory and legislative groups.

APPENDIX

Minority Statement by Dr. Burger

Since I feel that this matter is so important I am obliged to submit a minority view which is that the following explicit language should be a part of the final report:

> The finding of histologically benign tumors—especially in experimental animals—presents a particular challenge. In the first instance, the judgment of malignancy, resting on morphological cellular characteristics, is made through the exercise of sound judgment and experience. Secondly, the inference of progression from histologically benign tumors to malignant neoplasia is sometimes plagued with uncertainty. Transformations are known to occur—more often in some organ systems and cell types than in others. Appropriate judgment should rest on detailed knowledge and experience as to what these various probabilities are in each case. Finally, this picture is particularly complicated when trying to infer human experience from the finding of histologically benign tumors in experimental animals. Confirmatory investigations should be sought in these cases wherever appropriate.

Edward J. Burger, Jr., *M.D., Sc.D.*

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