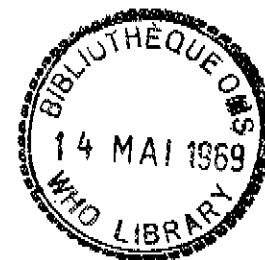




WORLD HEALTH ORGANIZATION  
ORGANISATION MONDIALE DE LA SANTÉ

INDEXED



PRINCIPLES FOR THE TESTING AND EVALUATION  
OF DRUGS FOR CARCINOGENICITY

Report of a WHO Scientific Group

Geneva, 2-7 December 1968

DSE/69.3

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation de l'Organisation Mondiale de la Santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

Members:\*

Professor I. Berenblum, Head, Department of Experimental Biology, The Weizmann Institute of Science, Rehovot, Israel (Chairman)

Dr L. Golberg, Research Professor of Pathology, Institute of Experimental Pathology and Toxicology, Albany Medical College of Union University, Albany, N.Y., United States of America (Rapporteur)

Dr F. J. C. Roe, Chester Beatty Institute of Cancer Research, Royal Cancer Hospital, London, England (Rapporteur)

Professor R. Schindler, Department of Pathology, University of Berne, Switzerland

Professor P. Shubik, Director, The Eopley Institute for Research in Cancer, University of Nebraska College of Medicine, Omaha, Nebr., United States of America

Professor H. Terayama, Director, Physiological Chemistry Laboratory, Zoological Institute, University of Tokyo, Japan

Dr B. Terracini, Institute of Pathological Anatomy, University of Turin, Italy

Professor R. Truhaut, Director, Toxicological Research Centre, Faculty of Pharmacy, University of Paris, France (Vice-Chairman)

Representatives of other organizations:

Dr J. F. Delafresnaye, Director, Geneva Office, International Union Against Cancer, Switzerland

Secretariat:

Dr A. C. Frazer, Director-General, British Nutrition Foundation, London, England (Consultant)

Dr H. Friebel, Chief Medical Officer, Drug Safety, WHO (Secretary)

Dr H. Halbach, Director, Division of Pharmacology and Toxicology, WHO

Dr H. Immich, German Cancer Research Centre, Heidelberg, Federal Republic of Germany (Consultant)

Professor P. N. Magee, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, England (Consultant)

Dr G. E. Paget, Managing Director, Smith, Kline and French Laboratories Ltd., Welwyn Garden City, Herts., England (Consultant)

Dr L. Tomatis, Chief, Chemical Carcinogenesis Unit, International Agency for Research in Cancer, Lyon, France

---

\* Unable to attend: Dr N. P. Napalkov, N. N. Petrov Research Institute of Oncology, Leningrad, USSR

CONTENTS

|   | <u>Page</u> |
|---|-------------|
| 1. Introduction . . . . .                                 | 4           |
| 2. General considerations . . . . .                       | 5           |
| 3. Drugs to be tested . . . . .                           | 6           |
| 4. Methods of testing . . . . .                           | 7           |
| 5. Retrospective and prospective studies in man . . . . . | 13          |
| 6. Interpretation of results . . . . .                    | 14          |
| 7. Recommendations and research needs . . . . .           | 16          |
| Annex. Guidelines for pathological examination . . . . .  | 17          |

A WHO Scientific Group on Principles for the Testing and Evaluation of Drugs for Carcinogenicity met in Geneva from 2-7 December 1968. The meeting was opened on behalf of the Director-General by Dr H. Halbach, Director, Division of Pharmacology and Toxicology, who outlined the terms of reference for the present meeting as part of the WHO programme for the promotion of drug safety.

## 1. INTRODUCTION

In accordance with a resolution<sup>1</sup> adopted by the Seventeenth World Health Assembly, WHO has convened several Scientific Groups<sup>2</sup> to assist in "the formulation of generally acceptable principles and requirements for the evaluation of the safety and efficacy of drugs". The first of these Groups dealt with general toxicity, but did not give detailed attention to the testing of substances of possible therapeutic interest for genetic and carcinogenic effects. The present Scientific Group was convened to discuss the latter topic and, in keeping with the view that it is "inadvisable to establish and prescribe rigidly formulated regulations specifying in detail the tests to be performed",<sup>3</sup> endeavoured to lay down the basic principles that should be applied to testing and evaluating the carcinogenic risks of drugs.<sup>4</sup>

Considering that all drugs should be evaluated for possible carcinogenic action, the Group established a set of priorities for the undertaking of such evaluation. The different categories of priority are based on criteria such as chemical or biological properties known to be involved in carcinogenesis and the stage of experimental and clinical development that drugs have reached. Evaluation of the carcinogenic risk may or may not include tests in animals. In addition, the Group emphasize the fact that prospective epidemiological studies and human surveillance are essential for the adequate evaluation of certain drugs.

When reviewing the test methods available, the Group paid particular attention to the interpretation of the experimental production of local sarcomas.

An important conclusion of the Group was that the demonstration of the carcinogenicity of a given substance in an animal need not necessarily, and under all circumstances, preclude the use of the substance in human therapy, provided it is done under strict medical supervision.

<sup>1</sup> Resolution WHA17.39 (Off. Rec. Wld Hlth Org., 1964, No. 135, p.17).

<sup>2</sup> Previous Groups have dealt with the preclinical testing of drug safety, the testing of drugs for teratogenicity, and the clinical evaluation of drugs (Wld Hlth Org. techn. Rep. Ser., 1966, No. 341; 1967, No. 364; and 1968, No. 403, respectively).

<sup>3</sup> WHO Regional Office for Europe (1964) Symposium on the toxicology of drugs, Copenhagen (mimeographed document). A limited number of copies of this document is available, to persons officially or professionally concerned with this field of study, on request to the WHO Regional Office for Europe, Copenhagen, Denmark.

<sup>4</sup> In carrying out this task, the Group took into consideration the following publications: Wld Hlth Org. techn. Rep. Ser., 1961, No. 220; Roe, F. J. C. (1966) Clin. Pharmacol. Ther., 7, 77; International Union against Cancer (1967) Potential carcinogenic hazards from drugs. /Proceedings of a symposium, Paris, 1965/, Berlin, Heidelberg & New York, Springer (IUCC Monograph series, vol. 7); Weisburger, J. H. & Weisburger, E. K. (1967) Meth. Cancer Res., 11, 307; European Society for the Study of Drug Toxicity (1964) Evaluation of the potential carcinogenic action of a drug, Amsterdam, Excerpta Medica Foundation (Proceedings, vol.3); and International Union against Cancer (1969) Carcinogenicity testing, Geneva (IUCC Technical Report No. 2).

## 2. GENERAL CONSIDERATIONS

For the purposes of this report, "a drug" is defined as "any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient".<sup>1</sup>

The use of agents or devices applied to, or introduced into, the body for prosthetic or contraceptive purposes is increasing. Such agents and devices are manufactured from various materials, often of novel composition, and although many of the factors considered in this report are applicable to them, the question of their potential carcinogenicity is not covered. Accordingly, it is recommended that consideration be given to arranging the discussion of these issues by a group of experts.

The Group found it necessary to draw a distinction between drugs already in general use and those not yet on the market. Relatively few of the very large number of chemical substances already in use as drugs have so far been tested for carcinogenicity. Priorities for the testing of such drugs are considered below (Section 3).

Several chemical compounds are now known to give rise to cancer both in animals and in man. These compounds have been widely studied and are well-documented. Amongst them are several therapeutic agents. Some of the early studies in carcinogenesis began with the observation of iatrogenic cancers, the most important of which resulted from exposure to ionizing radiation.

It has been reported that certain drugs, such as chlornaphazine and Thorotrast, have caused cancer in man following their administration as therapeutic agents. For some other drugs, such as diethylstilbestrol, phenylbutazone, and inorganic arsenicals, there is evidence suggestive of carcinogenicity in man. Certain other drugs, such as thiourea, isoniazid, and iron-dextran, are carcinogenic under some circumstances in experimental animals, but no evidence of cancer in man associated with the use of these substances has so far come to light. The lack of evidence of carcinogenicity in man of compounds known to be carcinogenic in animals results mainly from difficulties inherent in retrospective epidemiological studies. Since cancer induction in man may take several decades, it is possible for a drug to remain in widespread use before its carcinogenicity becomes apparent. In such circumstances properly planned prospective epidemiological studies are likely to provide the earliest proof of carcinogenicity in man.

In the past, most substances that are carcinogenic in man have been detected as such in man, and their action has been subsequently confirmed by animal studies. In the present state of surveillance of the use of drugs by man, tests in laboratory animals still provide the major safeguard. However, every effort should be made to obtain more data on the action of drugs in man. Only when the effects of an agent in man are both unusual and dramatic is the association between cause and effect likely to become obvious in the absence of a system of surveillance. Even when the association is obvious, its discovery is liable to be delayed. If the effect of a given drug were to induce a type of cancer that already occurs commonly, the effect would certainly not be detected without adequately planned human surveillance.

The problem of evaluating the carcinogenic hazard presented by food additives was reviewed by a Joint FAO/WHO Expert Committee in 1960.<sup>2</sup> More recently, a WHO Scientific Group discussed the significance of local sarcomas produced by the subcutaneous injection of food additives.<sup>3</sup> Despite the similarities between tests for carcinogenicity of drugs and of food additives, the present Group considered it desirable to review the problem of evaluating the carcinogenic hazard of drugs in the light of recent experience and advances in knowledge. Furthermore, although the principles of assessing the carcinogenicity of drugs and of food additives are similar, the application of the findings to the evaluation of the hazard to man is based on rather different considerations.

<sup>1</sup> Wld Hlth Org. techn. Rep. Ser., 1967, No. 341, p. 7.

<sup>2</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220.

<sup>3</sup> Wld Hlth Org. techn. Rep. Ser., 1967, No. 348, p. 19.

In any evaluation of the safety of a drug, the conditions of its intended use must be clearly defined, and the assessment of carcinogenic hazard should take into account the recommended uses of the drug. If these uses are altered or extended, reassessment becomes necessary.

The possibility of carcinogenic hazard, and therefore the need for assessment in this respect, exist in relation to all drugs. Confident prediction of the carcinogenicity or non-carcinogenicity of a given drug is not possible on the basis of its chemical structure or biological activity, although such properties may indicate that high priority should be given to the testing of a given product. A lack of evidence of tumour induction is the most reliable criterion of noncarcinogenicity; data from observations on man are rarely adequate and evidence from experiments on laboratory animals can usually be obtained more quickly.

### 3. DRUGS TO BE TESTED

#### 3.1 Testing and evaluation

Throughout this report, a distinction is drawn between testing a drug for carcinogenicity in animals and evaluating its carcinogenic hazard for man. Such evaluation may be based on the results of an experimental study in animals or on other information, such as the characteristics of the drug, the manner in which it is likely to be used, and epidemiological evidence. These considerations may indicate that experimental studies in animals are unnecessary for a given drug.

#### 3.2 Priorities

Evaluation of carcinogenic hazard to man is essential for all drugs. This evaluation will determine whether experiments in animals should be carried out. When experiments in animals are to be carried out, different priorities for such tests can be established.

There are four stages in the development and introduction of a new drug: animal studies, initial studies in man, therapeutic trials, and surveillance after the drug is marketed (which nowadays is carried out over a period of at least two years). Some drugs should be tested for carcinogenicity during the first of these stages, whereas others may be studied during the later stages. Evaluation should be made at each stage, taking into account any new information that may have been obtained.

Deciding on the stage at which a drug should be tested for carcinogenicity is necessarily complex and is considered in the following sections.

##### 3.2.1 Drugs to be tested before they are given to man

A compound may present so obvious a risk of carcinogenicity that its administration to man, either patient or volunteer, would entail an unacceptable risk unless experiments on animals showed the compound to be probably free from cancer-inducing activity. In special cases, however, the therapeutic need might be so serious that delay would be unjustified, and testing for carcinogenicity prior to clinical trials in patients might be waived.

Some categories of compound that must be tested at this stage are noted in the two following sections.

Drugs chemically related to known carcinogens. The variety of chemical structures shown to be associated with carcinogenic activity continues to increase. It is impossible to lay down rules as to the degree of similarity in chemical structure that should exist for a drug to be considered to resemble a known carcinogen. Testing is essential when both chemical and biological properties of a new compound resemble those of a carcinogen, or when metabolites with similar properties are formed from both the compound and a known carcinogen.

Drugs with certain specific biological effects. Many known carcinogens damage rapidly-growing tissues (e.g., the haemopoietic system and the intestinal mucosa) in relatively short

experiments, and some affect mitosis. When a new substance is shown to have similar biological effects, carcinogenicity tests are essential.

### 3.2.2 Drugs to be tested during clinical trials

There may be special reasons why carcinogenicity testing should not be delayed until after release for marketing. For instance, some drugs that are likely to be administered for long periods of time or to certain types of patient (e.g., newborn babies and pregnant or lactating women) should be tested for carcinogenicity during the clinical trial stage if they have not been previously tested.

### 3.2.3 Drugs to be tested after the decision to release for marketing

Final determination of the need for carcinogenicity testing should be made for every drug before it is released for marketing, when all the information from studies in animals and in man is available. Carcinogenicity testing could still be carried out during the period of special surveillance that follows release of the drug to the market, and this should be done unless a carcinogenic hazard can be ruled out with reasonable confidence.

### 3.2.4 Drugs on the market that should be tested

Many drugs on the market have not been subjected to carcinogenicity tests, and their possible carcinogenicity for man has not been evaluated. Priorities for the testing of such drugs are governed by the same considerations as apply to new drugs. Epidemiological studies may sometimes provide evidence of the carcinogenic hazard (or lack of it) arising from the use of a given drug.

It is beyond the scope of this report to specify which drugs should be tested or further evaluated or who should conduct such tests. It is suggested that a meeting of experts might be convened to consider these problems.

## 4. METHODS OF TESTING

### 4.1 Long-term animal tests

Although the ways in which they are interpreted may differ, tests for the carcinogenicity of drugs are similar to those for the carcinogenicity of other substances, such as food additives and pesticides. Long-term animal tests for carcinogenicity have been discussed in a number of publications,<sup>1</sup> but the Group felt that experience and advances in knowledge during recent years make it necessary to review some aspects of the design of such tests.

#### 4.1.1 Identity and purity of material under test

The composition and formulation of all drugs must be fully known and adequate specifications appropriate to the methods by which they are manufactured should be available.<sup>2</sup> Adequate specifications are especially necessary for products of unknown composition (e.g. plant or animal tissue extracts). Similar considerations apply to food additives and have been discussed elsewhere.<sup>3</sup>

<sup>1</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220; Clayson, D. N. (1962) Chemical carcinogenesis, London, Churchill, pp. 55-100; US National Research Council, Food Protection Committee (1960) Problems in the evaluation of carcinogenic hazard from use of food additives, Washington, D.C. (Publication 749); Hueper, W. C. & Conway, W. D. (1964) Chemical carcinogenesis and cancers, Springfield, Ill., Thomas, pp. 403-604; European Society for the Study of Drug Toxicity (1964) Evaluation of the potential carcinogenic action of a drug, Amsterdam, Excerpta Medica Foundation; International Union against Cancer (1969) Carcinogenicity testing, Geneva (IUCC Technical Report No. 2).

<sup>2</sup> Wld Hlth Org. techn. Rep. Ser., 1967, No. 341, p. 11.

<sup>3</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220; 1967, No. 348.

#### 4.1.2 Metabolism of the drug

The choice of animals for toxicological investigations aimed at predicting possible hazards of a given drug for man should take into account the ways in which the drug is metabolized in different animal species, including man. This is facilitated by the fact that such studies are often undertaken early in the development of a new drug. However, if a given drug falls into the group whose carcinogenicity must be tested before administration to man, the test animals must be chosen without knowledge of the way in which the drug is metabolized in man. If administration to man is permissible later, it is important to compare the ways in which the drug is metabolized in the test animals and in human subjects before any final evaluation of carcinogenic hazard is made.

#### 4.1.3 Animals used for carcinogenicity tests

Rodents. Rats, mice, and hamsters are generally regarded as being suitable for carcinogenicity tests. In the past, rats and mice have been used most frequently. There are indications that hamsters may be more suitable than either rats or mice for testing certain aromatic amines. The guinea-pig is known to be refractory to the action of some carcinogens and is considered unsatisfactory for routine test purposes. The rabbit is of limited use in carcinogenicity tests. The desert rat and steppe lemming may prove useful for test purposes in the future, but information on their suitability is limited at present.

Dogs. The dog is still recommended for testing suspected bladder carcinogens of the aromatic amine group, though studies now in progress may show that it is not indispensable for this purpose. Periods as long as seven years have been found necessary for tumour production by bladder carcinogens in the dog. The use of the dog for carcinogenicity tests in general is not practical.

Monkeys. Recent studies indicate that monkeys are sensitive to a variety of carcinogens and are likely to prove valuable for the evaluation of carcinogenicity, particularly in the testing of certain hormonal preparations.

Other non-rodent mammals. No suitable non-rodent mammal can at present be recommended for general carcinogenicity testing. Such an animal is urgently required.

Trout. Although the trout has been reported to be susceptible to the induction of hepatoma, its use for the testing of drugs is likely to be limited owing to the lack of background pharmacological information on this fish.

Birds. Although birds - particularly budgerigars, ducks, hens, and quail - are known to be susceptible to carcinogens, more background information is needed before they can be recommended for general testing purposes.

Strains. Inbred and outbred strains of rats and mice are readily available, but only inbred mice have been studied extensively. More recently, inbred hamsters have become available. For most carcinogenicity tests closed colonies of outbred rodent stocks are satisfactory. However, the effects of carcinogens can sometimes best be detected in certain inbred strains. Information on special strain characteristics should be taken into account in designing tests of drugs whose structure or biological activity are similar to those of known carcinogens.

Pathogen-controlled animals. In all long-term tests, such as those for carcinogenicity, the health of the animals is of paramount importance. A technique for reducing the risk of microbial and parasitic disease has been widely used during the past decade. Animals are delivered by Caesarian section and then reared under conditions that protect them and their progeny from contact with infectious agents. Colonies of animals so established and maintained - referred to as "pathogen-controlled" animals - can be virtually freed from certain infections - e.g. the rat can be freed from chronic pneumonia and the mouse from ectromelia, Pseudomonas infection, and salmonellosis. Pathogen-controlled animals tend to

be healthier and live longer than animals in unprotected colonies, but they are not immune to infection. Whatever the animals used in carcinogenicity tests, it is important to know and record the conditions under which they have been bred and reared, and any infections and infestations that may be present in them.

Newborn and infant animals. The use of newborn animals may offer advantages - e.g. positive results may be evident early and may be obtained with only small amounts of the test material. However, these are insufficient reasons at present to recommend the use of newborn or infant animals rather than adult animals for carcinogenicity testing. Restricting the administration of the agent being tested to the first few days or weeks of life, may result in inadequate exposure and there may under these circumstances be no grounds for accepting a negative result even if the period of observation covers the major part of the normal life span of the test species.

#### 4.1.4 Animal husbandry

Diets. The presence of naturally-occurring carcinogens, particularly aflatoxins, in composite animal diets has necessitated the introduction of semisynthetic diets for use in studies of such compounds. This, in turn, has led to a re-examination of the types of diet fed to animals in toxicology studies generally. It is apparent that there is no control of naturally-occurring carcinogens, pesticide residues, and intentional and unintentional food additives in most commercially available diets. Properly controlled carcinogenesis studies require a defined diet in which the impurities are known. At this time no semisynthetic diet can be recommended, but it is to be hoped that research will lead to the development of nutritionally adequate defined diets that can be made available at a reasonable cost.

Air. The air in most urban areas is contaminated with known chemical carcinogens. No entirely satisfactory filtration system can at present be recommended for animal quarters, but it is to be hoped that the situation will be improved as the result of further research.

Pesticides. All those concerned with the management of experimental animal stocks should be cautioned against the excessive spraying of animal quarters with pesticides, anti-septics, or other chemicals. Apart from the possibility that such agents may be carcinogens, they may alter the response of animals to drugs.

Water. There appears to be no reason for recommending the use of distilled water in preference to tap water for animal consumption. Indeed, distilled water and chemically-treated water may often be less satisfactory than tap water, since they may contain adventitious contaminants.

The use of hypochlorite in drinking water has been recommended for the control of Pseudomonas infection in mice. Since there are alternative means of controlling this infection - e.g. the use of pathogen-controlled stocks - and since hypochlorite may interfere with the action of carcinogens, this practice is not recommended.

#### 4.1.5 Experimental plan

Number of animals. The first step in designing any experiment is the precise formulation of the question that it is intended to answer. At this stage the following statistical information should be available:

- (1) the expected incidence of tumours in the control group, based on previous experience of the animals to be used;
- (2) the expected death rate in the entire experiment from causes other than tumours;
- (3) the smallest difference in incidence of tumours between the various test and control groups that the experiment should be designed to detect;

(4) the degree of confidence with which this difference should be detected; and

(5) whether it is sufficient to consider only qualitative data (the presence or absence of a tumour in given animals) or whether quantitative aspects, particularly the time of appearance of tumours, must also be considered.

Given this information, a statistician is in a position to calculate the minimum number of animals that each group must contain in order that the questions posed may be answered. It may be advisable to exceed this minimum group size in order to allow for unforeseen causes of death, including toxicity of the test compound.

In certain circumstances it may not be possible to adopt the above procedure. If so, guidance on the numbers of animals to be used can be obtained elsewhere.<sup>1</sup>

Sex. With the exception of tests for hormone-related carcinogenicity, it is essential that studies be conducted on both sexes, since there are many examples of sex-related differences in response to known carcinogens.

Number of species. At least two species should be used.

Control groups. Although accumulated background information about animal stocks is of great importance, control groups of untreated animals are always necessary. When the nature of the vehicle in which the drug is administered so demands, a control group receiving it alone should be included.

Animals should always be randomly allocated to the different groups within an experiment.

When carcinogenicity testing of a given drug is specially indicated by the similarity of its chemical structure or biological effects to those of a known carcinogen, a positive control group treated with the latter should be included in the experimental design. In other cases, the inclusion of a positive control group may be desirable merely to establish that the test animals are capable of responding to a carcinogenic stimulus under the general conditions of the experiment. However, when carcinogenicity tests are conducted on animals of known susceptibility, the inclusion of a positive control group may be superfluous. If a known potent carcinogen is used in positive control experiments, precautions must be taken to prevent any hazard to personnel.

Duration of experiment. In general, it is best to start administration of the drug as soon as possible after the animals are weaned. There are disadvantages in carrying on studies to the end of the natural life span, although this seems to be right in principle. The experimental period is commonly terminated before the end of the natural life span, but it should not be reduced to less than two years for rats and hamsters or less than 18 months for mice.

Dose levels. Owing to the great importance of establishing dose-response relationships in carcinogenicity tests, the use of at least three dose levels is essential. The fifth report<sup>2</sup> of the Joint FAO/WHO Expert Committee on Food Additives gives useful guidance on the choice of appropriate dosage levels. Certain drugs may present particular problems if their

<sup>1</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220; Weisburger, J. H. & Weisburger, E. K. (1967) Meth. Cancer Res., 1, 307; International Union against Cancer (1969) Carcinogenicity testing, Geneva (IUCC Technical Report No. 2).

<sup>2</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220.

early and late toxic effects make it impossible to administer large doses. This difficulty can sometimes be avoided by the use of divided daily doses. The highest dose level used should be within the toxic range, but should be consistent with the prolonged survival of a majority of the animals. The lower levels should permit the animals to survive in good health for their natural life span or until tumours develop.

#### 4.1.6 Experimental procedures

Choice of vehicle. The choice of solvent or other vehicle for administering the drug is important in carcinogenicity studies. The carcinogenic potential of the vehicle itself or of any impurities it may contain must be fully known or investigated. Strict specifications are needed to ensure that vehicles of adequate purity are used.

Route of administration. Two factors determine the choice of the route of administration. The first is the route used, or intended to be used, clinically, since it is important that a drug be given experimentally by the same routes as those used clinically. The second factor is the need to ensure that tissues and organs in the experimental animal are exposed to concentrations of the drug and its metabolites at least as high as, and preferably higher than, those to which human tissues are exposed. Tissue concentrations of the drug should be ascertained unless other evidence makes this unnecessary. With drugs that are administered orally to man, adequate tissue concentrations are usually produced by giving them orally to experimental animals. Administration to animals should be by gavage whenever possible.

When appropriate tissue concentrations are not attained by the route of administration used clinically, another route that produces such concentrations must be sought. This requirement may give rise to severe difficulties when the subcutaneous route is used (see Section 6.1.1); in such circumstances consideration should be given to the intraperitoneal route.

The fifth report of the Joint FAO/WHO Expert Committee on Food Additives discusses the question of whether, in testing food additives for carcinogenicity, two routes of administration should be used,<sup>1</sup> and recommends that, in special circumstances, "a suitable parenteral route of administration" be used.<sup>2</sup> When the route used clinically can be employed experimentally, giving rise to appropriate tissue concentrations, the use of a second route of administration usually offers little advantage and may cause difficulties. Consequently, the Group does not recommend the routine use of two routes of administration in testing drugs for carcinogenicity. Additional effort is most effectively directed to the achievement of a high standard of performance in a test involving a single route of administration.

Frequency of administration. The frequency of administration of the drug should be related to the regime of clinical administration. This may mean that the drug must be given to animals daily, but in special circumstances administration on five or six days per week is acceptable.

#### 4.1.7 Pathological examination

Full necropsies should be performed on all animals, including those that are decomposed. Decomposition and cannibalism can be minimized by daily examination of the animals. Macroscopic observations should be made by persons trained in pathological techniques and microscopical studies should be carried out by experienced pathologists.

<sup>1</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220, p. 16.

<sup>2</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220; Weisburger, J. H. & Weisburger, E. K. (1967) Meth. Cancer Res., 1, 307; International Union against Cancer (1969) Carcinogenicity testing, Geneva (IUCC Technical Report No. 2).

The procedure for gross examination and a list of the organs that should be examined macroscopically are given in the Annex. Although it is desirable that the same organs be examined microscopically, this is often impractical. A reasonable routine procedure is to examine microscopically certain organs in all animals (including lungs, liver, spleen, urinary bladder, and kidneys) as well as those showing gross alterations, and to preserve all other organs and the carcass in fixative until the end of the experiment.

When experiments include studies of the effects of different dose levels, and no evidence of carcinogenicity emerges from a comparison of the high dose group and the control group, histological examination of animals in the intermediate and low dose groups may be unnecessary.

The standardization of nomenclature in animal pathology would be of great help to carcinogenicity studies.

#### 4.1.8 Presentation of results<sup>1</sup>

Results must be presented in a clear and organized form and in adequate detail. The information that should normally be included in reports has been listed elsewhere.<sup>2</sup>

Negative as well as positive results should be available to scientists. Since it may be difficult to obtain publication of negative results, it is recommended that WHO consider the possibility of establishing a mechanism for the central collection of the results of carcinogenicity tests of drugs and for the dissemination of this information.

#### 4.1.9 Testing of hormonal contraceptives

A number of synthetic or semisynthetic substances are used in hormonal contraceptive preparations, and these may be taken for a substantial part of the life span. The contraceptive activity of these substances is related to the hormonal effects they produce in females. However, they might be used in the future for other purposes in males as well as in females. In view of their long-term use under relatively little medical supervision, it is advisable that they be tested for carcinogenicity in species normally used for this purpose. For the reason given above, both male and female animals should be studied.

Since variations in hormonal action in different species might affect the results of carcinogenicity tests, it is advisable to study this question in an animal that displays hormonal effects similar to those brought about in man. Suitable female primates are the animals of choice for this purpose. Prospective epidemiological studies in man are also of great importance in this area.

#### 1.2 Rapid tests

The long duration and cost of the carcinogenicity tests now in use make it desirable to develop reliable methods that are quicker and cheaper. Several attempts have been made to discover correlations between the ability of chemicals to induce tumours and to elicit other biological effects that appear sooner than cancer. However, the methods developed so far cannot be recommended as a substitute for carcinogenicity tests, despite their interest and value for research purposes.

---

<sup>1</sup> This subject is dealt with in considerable detail in International Union against Cancer (1969) Carcinogenicity testing, Geneva (IUGC Technical Report No. 2).

<sup>2</sup> Wld Hlth Org. techn. Rep. Ser., 1961, No. 220, p. 33.

The implantation of the test material together with tissues derived from embryos has been used as a rapid test, but its value for testing drugs is not known.

Although some compounds are both carcinogenic and teratogenic, the two types of activity are not consistently related. Consequently, the absence of teratogenic effects does not imply noncarcinogenicity.

#### 4.3 In vitro tests

Cellular transformation and carcinogenesis have been studied extensively in cell or organ cultures in recent years, although such studies have not resulted in the detection of a previously unsuspected carcinogen. Certain noncarcinogenic chemicals are converted metabolically in vivo to derivatives with carcinogenic activity. However, such chemicals may not be metabolized in cell cultures, giving rise to false negative results. Conversely, carcinogens that are rapidly inactivated in vivo may give rise to false positive results in cell cultures.

Two further serious limitations of in vitro tests are the uncertainties involved in interpreting positive findings and the risk that negative findings may be uncritically accepted as evidence of noncarcinogenicity.

### 5. RETROSPECTIVE AND PROSPECTIVE STUDIES IN MAN

The possible carcinogenic action of a drug in man can be studied retrospectively or prospectively. The first indications of an association between the use of certain drugs and the subsequent occurrence of cancer have emerged from retrospective studies. Although the usefulness of this method cannot be overlooked, it involves many difficulties and has inherent flaws. Such studies often lead to an assumption of causality as a result of misinterpreting an incidental association. Furthermore, both interviewers and patients may have too great a knowledge of the disease under study to provide unbiased answers.

The method of prospective study has been evolved to overcome these and other difficulties. A notable example of the success of this method was the confirmation of the association between cigarette smoking and bronchogenic carcinoma in man, which had previously been suggested by retrospective studies. However, prospective studies also involve difficulties, as follows:

(1) It is conceivable that a particular disease process, rather than the drug used for its treatment, may be causally related to the occurrence of a cancer. The differentiation of such factors is a common complexity of prospective studies.

(2) It is often impossible to have an appropriate control group that is not treated with the drug (e.g. tuberculosis patients not treated with isoniazid).

(3) The effects of the drug under study may be difficult to distinguish from those of (a) other drugs given at the same time, (b) known carcinogenic factors such as smoking, (c) dietary factors, (d) therapeutic irradiation, and (e) various environmental factors known to influence the genesis of cancer in man. In this respect, early studies were at a great disadvantage in being limited to a simple comparison of incidence; the use of multivariate analysis has greatly increased the power of statistics.

In addition to prospective studies involving selected drugs another method can be used to provide a broader check. If information can be obtained on the real consumption of drugs by the entire populations of countries, comparisons can be made between trends in cancer incidence and trends in the consumption of drugs.

In certain countries systems of medical record linkage are being developed. The introduction of electronic computers has made this method a practical approach to the detection of carcinogenic and other hazards.

## 6. INTERPRETATION OF RESULTS

### 6.1 Type of response

The response of test animals to carcinogens may take one of several forms: (1) an increased incidence of one or more of the tumour types noted in the controls; (2) the occurrence of tumours earlier than in the controls, without increased incidence; (3) the development of types of tumour not seen in the controls (this may or may not be associated with an overall increase in the number of tumours seen in the controls); and (4) a multiplicity of tumours in individual animals, the incidence in terms of tumour-bearing animals being the same. Furthermore, the tumours seen may be benign or malignant, or tumours of both categories may be present.

All these types of response are at present used to classify chemicals as carcinogens, no distinction being made between them, although it is clear that different types of response may give rise to different degrees of concern. The aim should be to study these problems in detail so that, ultimately practical recommendations on any substance can be based on consideration of both the hazards and the benefits that might result from its proposed use.

The problem of the carcinogenicity of isoniazid illustrates many of the complexities discussed in this report. Isoniazid has been shown to increase the incidence of tumours of specific types in only one animal species, the mouse. In view of the importance of this drug in the chemotherapy of tuberculosis, its withdrawal could not be recommended on the basis of this evidence from animal studies. Clearly, if the drug had been shown to induce a wide variety of tumours in several species, a different decision would have been indicated. It is recommended that efforts be made to organize prospective studies in human beings treated with isoniazid and that more extensive studies in animals be undertaken.

#### 6.1.1 Sarcoma induction in rats and mice

The significance of the occurrence of local sarcomas following subcutaneous injection was considered in 1966 by a WHO Scientific Group, who recommended "that for the routine testing of food additives and contaminants, the subcutaneous injection test should be considered inappropriate unless special conditions, such as lack of absorption from the gastrointestinal tract under conditions of routine feeding to experimental animals, demand additional studies".<sup>1</sup>

Since the subcutaneous or intramuscular route may be employed clinically for the administration of drugs to man, it is necessary to discuss the implications of subcutaneous sarcomas produced experimentally by using these routes in rats and mice.

When administration by the subcutaneous or intramuscular route is associated with the development of tumours at sites distant from the site of injection, the criteria of carcinogenic response defined above are applicable, whether or not local sarcomas also develop. Consequently, the point at issue is the case in which only local tumours develop. Fibromas or sarcomas at the injection site may be the consequence of introducing carcinogens subcutaneously into rats or mice. Such tumours may or may not result from the operation of physical factors<sup>2</sup> - i.e., physical properties of the test material that are unrelated to any chemical carcinogenic potential it may possess. Tumour induction may follow the introduction of chemically unreactive materials in solid form or the local deposition in insoluble state of a substance introduced in solution. In such cases physical characteristics - e.g., the dimensions of the material, whether or not it softens at body temperature, and the smoothness of its surface - may determine whether or not tumours appear.

<sup>1</sup> Wld Hlth Org. techn. Rep. Ser., 1967, No. 348, p. 19.

<sup>2</sup> See Wld Hlth Org. techn. Rep. Ser., 1961, No. 220, p. 16.

It has been suggested that one or more properties of injected solutions (e.g., hypertonicity, pH, and surface activity), as well as factors affecting the rate at which such solutions are absorbed from the injection site, may determine whether or not a tumour is formed. When testing drugs that are administered to man by subcutaneous or intramuscular injection, there is no simple way of selecting the appropriate dose to be administered to a small animal. The injection of large doses in relation to the body weight of the animal (particularly of substances that accumulate at the injection site), or the use of relatively concentrated solutions, may create physical effects whose consequences may be different from those produced in man.

Certain difficulties inherent in the use of the subcutaneous route may be avoided to some extent by distributing the material between multiple injection sites to reduce the risk that cumulative tissue damage at one site may influence sarcoma induction.

Recognition of the part that may be played by the physical properties of a substance in the production of local sarcomas in the rat and mouse renders it difficult to interpret the significance of the induction of such tumours, especially if the substance is not carcinogenic when tested by other routes of administration. Detailed study of the nature and evolution of local tissue changes at the site of injection and of the mechanism by which they arise may help to resolve the problem.

If additional studies, including the use of other parenteral routes, do not provide sufficiently clear evidence of safety, and if suspicion of the carcinogenic hazard of a subcutaneously administered drug rests solely on its induction of local sarcomas, the Group recommends that the drug may be used clinically, provided arrangements are made for surveillance.

#### 6.2 Dose-response relationships

As with all chemical substances that give rise to biological effects, it is often possible to show that a positive response in a carcinogenicity test is dose-related. The Group considers that the demonstration of a dose-response relationship in carcinogenicity tests of a drug should be taken fully into account in evaluating the balance of benefit and risk associated with the use of the drug in patients.

The dosage of known carcinogens required to bring about a positive response varies considerably. For example, aflatoxin can cause cancer at a daily dose level of a few  $\mu\text{g}$  per kg of body weight, whereas the dosage of diethylstilboestrol required to produce such an effect is about 1000 times this level. Many factors may modify these dose requirements, but this does not alter the fact that gross differences exist between the dose-response relationships of different carcinogens.

#### 6.3 Evaluation of potential hazard to man

If there is evidence of a possible carcinogenic hazard from food additives and ingredients of cosmetics, such substances should not be used. This is not necessarily so with drugs, since circumstances exist in which it is proper to use a drug that has been shown to be carcinogenic experimentally. Each drug must be evaluated individually. This Group does not wish to lay down a set of rules for such evaluation - and, indeed, could not do so - but some general guidance on the factors that should be considered is given below.

Some types of response may suggest that long continued exposure to a given carcinogen is required before a positive response becomes evident. Clearly, this would cast doubt on the wisdom of using such a compound as a drug for long periods of time. It is less certain that it should prevent the use of the drug on a single occasion (or a small number of occasions) in any one individual. Other types of response may suggest that a hazard will arise only in particular circumstances of use - e.g., subcutaneous injection (see section 6.1.1). Such a response should not necessarily preclude all other uses of the drug.

Drugs are used medically for a wide range of conditions from the trivial to the serious, and a given drug may be used for both trivial and serious conditions. Drugs may be given to an individual only once, or several times a day for many years. Many drugs already on the market have not been evaluated for carcinogenesis. Serious hazards may be associated with drugs available without medical prescription. The Group recommends that any drug that has been shown to be a carcinogen experimentally be used only under strict medical supervision.

## 7. RECOMMENDATIONS AND RESEARCH NEEDS

### Animal studies

- (1) The improvement of methods for comparative study of the metabolism of drugs in animals and man.
- (2) Extension of the use of computer techniques in carcinogenicity studies.
- (3) The development of useful short-term carcinogenicity tests.
- (4) Investigation of the suitability of nonrodent mammals for carcinogenicity testing.
- (5) Investigation of modifying factors in carcinogenesis.
- (6) Investigation of a possible association between particular types of pharmacological action and the manifestation of carcinogenicity.
- (7) The development of defined diets for laboratory animals used in carcinogenicity studies.
- (8) Investigation of the possible carcinogenicity of agents and devices applied to, or introduced into, the body for prosthetic or contraceptive purposes.

### Human studies

The promotion and co-ordination of prospective epidemiological studies of the carcinogenic hazards presented by certain drugs, particularly hormonal contraceptives. National bodies should take all possible measures to facilitate such prospective studies, particularly by collecting data on drugs.

### Evaluation of carcinogenic hazards

Detailed examination of the practical implications of the use of drugs known or suspected to be carcinogenic. A drug shown to be a carcinogen experimentally may, in certain circumstances, be used clinically but only under strict medical supervision. The decision on such issues should be made by individuals who are familiar not only with experimental carcinogenesis but also with the nature and use of drugs.

### Information services

- (1) Establishment of a register of drugs already on the world market, and of priorities for evaluating such drugs for carcinogenicity.
- (2) Improved dissemination of information on the carcinogenicity of drugs to all involved in this use.
- (3) Establishment of a comprehensive register of information on the negative and positive results of carcinogenicity tests carried out on all chemicals (not only drugs).

ANNEX

GUIDE LINES FOR PATHOLOGICAL EXAMINATION

Full necropsies should be performed on all animals, including those that die accidentally or are found dead and possibly decomposed. Complete pathological records should include details of the killing procedure. Observations may be hindered by advanced decomposition or cannibalism, but these eventualities can be largely avoided if animals are examined daily, sick animals being killed. Even when decomposition is too advanced for microscopic examination, useful data may be obtained from macroscopic observation. The person who performs the necropsy should be trained in the technique and should be aware of any pre-existing signs of disease in the animal undergoing necropsy.

A complete necropsy procedure is outlined below. However, it is realized that in the past less thorough examinations have led to the detection of carcinogens and that the availability of staff may not always permit such a detailed study. Full necropsy includes weighing the animal, careful external examination and evaluation of degree of decomposition. Attention should be given to lips, tongue, oral cavity, external ears, eyes, limbs, vagina and anus. Blood smears should be prepared from animals due to be killed. Each organ should be examined both in situ and after removal. A record should be made of macroscopic normality or abnormality.

The following organs and tissues should be examined: subcutaneous tissues; mammary tissue; salivary glands; axillary, inguinal and cervical lymph nodes; mesenteric, parrenal, lumbar and caudal lymph nodes; thymus and mediastinal lymph nodes; tongue; oesophagus; stomach; duodenum; small and large intestines; rectum; kidneys; ureters; bladder; urethra; larynx; trachea; lungs; liver and gall bladder; pancreas; spleen; thyroid; parathyroids; adrenals; testes; epididymes; seminal vesicles and coagulating glands; prostate (various lobes); paraurethral and preputial glands; ovaries; uterus; cervix uteri; vagina; eyes; lacrimal glands; brain and meninges; pituitary; spinal cord; and bone marrow.

In every laboratory that performs tests for carcinogens it is advisable for necropsies to be performed according to a standard procedure. A sequence of investigation that has been found convenient is described below, although it may be necessary to vary the procedure according to individual requirements:

- (1) subcutaneous tissue, mammary tissue, salivary glands and peripheral lymph nodes;
- (2) opening of the abdominal and thoracic cavities and over-all evaluation of changes;
- (3) removal of the intestine from the duodeno-jejunal junction to the rectum (the mesentery should be carefully dissected and the mesenteric lymph node observed);
- (4) removal of the adrenals;
- (5) removal of the stomach, duodenum, pancreas and spleen;
- (6) splitting of a femur and preparation of bone-marrow smears, using a fine paint brush;
- (7) removal of kidneys, ureters and urinary bladder; and distension of urinary bladder with fixative;
- (8) for the females, opening of the vagina, cutting through the pubis and removal of vagina, uterus, salpynges and ovaries;
- (9) for males, removal of testicles, seminal vesicles and prostate, paraurethral and preputial glands;
- (10) opening and removal of the rectum (cutting through the pubis in males);

- (11) removal of the liver, dissecting the lobes and opening the gall bladder in appropriate species (the liver should be removed with the entire diaphragm except when there are strong adhesions to the lung);
- (12) examination of retroperitoneal organs;
- (13) severing of the mandible in the midline; removal of the tongue with neck and chest organs en bloc; examination of the palate and rhinopharynx; examination of thymus, mediastinal lymph nodes, heart, lungs and thyroid;
- (14) opening and dissection of the oesophagus;
- (15) opening of the trachea and main bronchi;
- (16) cutting of the spine at the cervical vertebrae and lifting up so as to examine the subcutaneous tissue of the back;
- (17) opening of the spine (when the spinal cord is to be examined);
- (18) removal of the eyes and lacrimal glands;
- (19) opening of the skull; examination of the meninges; removal of the brain; observation of and removal of the pituitary (in small animals it may be convenient to remove the pituitary after a short period of fixation; the brain is best examined after at least 12 hours fixation);
- (20) when the animals are sacrificed, opening of the stomach, intestine, skull and spine should be the last operations to be performed in order to avoid delay before fixing other organs that undergo quick decomposition. In all cases the urinary bladder, and in some cases other hollow organs, should be distended with fixative before removal from the body, as a preliminary to further examination.

All organs and the carcass should be preserved in fixative until the end of the experiment. The kidneys, liver lobes and spleen should be cut before fixation. Shortly after fixation the brain should be examined by serial transversal sections and the spinal cord should be removed from the spine (the pituitary should also be removed if this has not been done earlier). If the sinuses and the nasopharyngeal and ethmoidal regions require examination, the skull should be submitted to rapid decalcification.

It is desirable that all organs be examined histologically, but this is often impractical. Routinely, the following organs should be examined: (1) all organs showing gross alterations, and (2) lungs, liver, spleen, kidneys and urinary bladder. Since the entire animal is preserved in fixative, the pathologist will be able to carry out histological examinations of other organs at any stage of the experiment, should this become desirable.