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**WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES**

No. 144

**PROCEDURES FOR THE TESTING
OF INTENTIONAL FOOD ADDITIVES
TO ESTABLISH THEIR SAFETY
FOR USE**

**Second Report
of the Joint FAO/WHO Expert Committee
on Food Additives**

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WORLD HEALTH ORGANIZATION

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GENEVA

1958

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva, 17-24 June 1957

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This report was originally issued in mimeographed form as document WHO/Food Add./14.

PRINTED IN SWITZERLAND

PROCEDURES FOR THE TESTING OF INTENTIONAL FOOD ADDITIVES TO ESTABLISH THEIR SAFETY FOR USE

Second Report of the Joint FAO/WHO Expert Committee on Food Additives *

INTRODUCTION

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 17 to 24 June 1957. The meeting was opened by the Deputy Director-General of the World Health Organization, Dr P. Dorolle, who also spoke on behalf of the Director-General of the Food and Agriculture Organization. Professor R. Blackwell Smith, jr, and Professor R. Truhaut were unanimously elected Chairman and Vice-Chairman of the Committee. The Committee consisted of ten members, five of whom were invited by FAO and five by WHO. Members of the staff of FAO and WHO acted as technical secretaries.

Background

In 1954 the Joint FAO/WHO Expert Committee on Nutrition at its fourth session^a reviewed the problems associated with the use of food

* The WHO Executive Board, at its twenty-first session, adopted the following resolution :

The Executive Board

1. NOTES the second report of the Joint FAO/WHO Expert Committee on Food Additives ;
2. THANKS the members of the Committee for their work ;
3. EXPRESSES its appreciation to the Food and Agriculture Organization for its collaboration ;
4. AUTHORIZES publication of the report ; and
5. EXPRESSES the wish that the necessary measures to implement the recommendations embodied in the report be taken in co-operation with FAO.

(Resolution EB21.R5, *Off. Rec. Wld Hlth Org.*, 1958, 83, 6)

^a *Wld Hlth Org. techn. Rep. Ser.*, 1955, 97 ; *FAO Nutrition Meetings Report Series*, 1955, No. 9

additives and recommended that a special conference should be convened by FAO and WHO to consider this question. The Joint Committee also suggested that the conference might consider the desirability of convening an expert committee to consider, among other things, the criteria to be used in assessing the toxicity of food additives. In accordance with these recommendations a Joint FAO/WHO Conference on Food Additives consisting of delegates from a number of countries as well as of representatives of organizations working in this field was held in Geneva in September 1955.^a

This conference, which was of an exploratory nature, gave special consideration to the work which FAO and WHO could usefully undertake. It recommended that expert committees or groups should be convened to formulate general principles governing the use of food additives and to consider the formulation of "suitable uniform methods for evaluating the safety of food additives". The Joint FAO/WHO Expert Committee on Food Additives, meeting in Rome in December 1956 to formulate general principles,^b recommended that toxicological procedures should be examined at an early date. The present report is the result of these recommendations.

Terms of reference

In their letters of invitation the Directors-General of FAO and of WHO stated that, in accordance with the recommendations referred to above the Committee should "consider procedures for the toxicological testing of intentional food additives". In the main the Committee restricted its discussions to "non-nutritive substances which are added intentionally to food, generally in small quantities, to improve its appearance, flavour, texture, or storage properties", as proposed by the Joint FAO/WHO Expert Committee on Nutrition in 1954.^c

1. GENERAL CONSIDERATIONS

This report is intended to give to those engaged in this field, in either a scientific or an administrative capacity, a general picture of the type of data that should be available about any additive before its use in food

^a *Wld Hlth Org. techn. Rep. Ser.*, 1956, 107; *FAO Nutrition Meetings Report Series*, 1956, No. 11

^b The present report should be read in conjunction with the first report of the Joint FAO/WHO Expert Committee on Food Additives (*Wld Hlth Org. techn. Rep. Ser.*, 1957, 129; *FAO Nutrition Meetings Report Series*, 1957, No. 15).

^c *Wld Hlth Org. techn. Rep. Ser.*, 1955, 97, 30; *FAO Nutrition Meetings Report Series*, 1955, No. 9, p. 30

is officially approved. There have been a number of publications on this subject.^{1, 4, 5, 7, 10, 11, 14}

Scientists working in this field have a responsibility to indicate what should be done to provide evidence upon which decisions on safe use may be made. A well-designed study of the reactions of experimental animals to the administration of food additives, or the processed food itself, can provide the evidence necessary for making such decisions. As information accumulates in the field of general physiology and biochemistry the effects of substances such as food additives will be progressively more clearly understood. The fact that additives may be taken over the greater part of a lifetime gives rise to concern lest such prolonged intake may produce reactions hitherto unsuspected. The possibilities that any such reaction may follow the ingestion of food additives can best be examined experimentally by feeding much higher doses of the material to animals during the greater part, or the whole, of their lifetimes. In the absence of any indication to the contrary, such prolonged tests must form a part of any experimental work designed to supply evidence in support of a claim for the safe use of a food additive.

It cannot be too strongly emphasized that, just as the conduct of the experiments designed to test safe use is the responsibility of the scientist, so also is the evaluation and interpretation of the data from these experiments. In order that sound decisions can be made regarding the use of food additives, the scientific data that have been assembled must be made available in a form that will allow independent scientists with the appropriate experience to make an adequate assessment of the findings. The decisions reached by expert groups in different countries with respect to any particular food additive may vary, since the relative importance of different aspects of the problem may be affected by the circumstances, dietary habits or legislative background of the community that the expert group serves.

No single pattern of tests could cover adequately, but not wastefully, the testing of substances so diverse in structure and function as food additives. The Committee considers that the establishment of a uniform set of experimental procedures that would be standardized and obligatory is therefore undesirable. Furthermore, it would not necessarily resolve the difficulties that have sometimes been encountered in reaching decisions on the safe use of food additives. For this reason the Committee concluded that it was only possible to formulate general recommendations with regard to testing procedures.

The Committee wishes to stress the fact that such recommendations do not eliminate the need to search for other methods that might be simpler and better adapted to the purpose in question. While there might be unanimity about the value of certain techniques, the satisfactory evolution of better methods depends upon the existence of full scientific freedom

in this field. At any time a rapid advance in any of the basic sciences might suggest new ways of conducting toxicological investigations. The scientist in the laboratory can make immediate use of such developments, but it would take longer for any modification indicated as being desirable to become incorporated in any officially recommended testing procedures. The Committee strongly emphasizes the need for supporting work in any field that might help in the improvement of the methods available for evaluation of the safe use of a food additive.^a

The general aim of the work for which guidance is offered in this report is that of establishing the "safe use" of a chemical; it is not confined to a study of toxicity *per se*, or of "toxicological procedures". Most would agree that almost any chemical can be harmful at some dose and safe at another, although some workers maintain that there can be no safe level of dosage for proved carcinogens.

The Committee confirms the statement made in the first report of this Expert Committee that, while "safety for use is an all-important consideration... it is impossible to establish absolute proof of the non-toxicity of a specified use of an additive".^b The occasional case of idiosyncrasy may illustrate this point. It is also important to remember another principle set forth in the same report, namely, that the use of the food additive shall be to the advantage of the consumer.

The procedures outlined in this report require all the resources of a well-equipped laboratory with an adequately trained staff having access to current scientific literature over a wide field. In particular it is essential that facilities exist for the housing of large numbers of small laboratory animals and for smaller numbers of other species. Competent staff in numbers sufficient for the proper care of the animals is also essential. The provision of such facilities on an adequate scale is very costly. These questions must be examined and adequate means made available before a laboratory is set up to undertake toxicological investigations. Preferably it should be situated in an environment where active research in allied fields is in progress.

Progress in work on food additives would be helped if more relevant experimental results were published in scientific journals. In many instances these results remain available only to a restricted group. If the co-operation of those sponsoring these investigations could be assured, the international organizations might consider the possibility of preparing lists of unpublished reports, with a view to making them more widely available, on request, to workers in this field.

^a The safety of materials such as pesticides and certain unintentional additives that find their way into food may be evaluated by similar methods.

^b *Wld Hlth Org. techn. Rep. Ser.*, 1957, 129, 11; *FAO Nutrition Meetings Report Series*, 1957, No. 15, p. 11

2. CHEMICAL AND PHYSICAL IDENTIFICATION

In its first report, the Expert Committee emphasized the need for specifications of identity of food additives in physical and chemical terms and recommended that FAO and WHO arrange by appropriate means for a comprehensive review of this subject.^a The Committee endorses this recommendation and further recommends that a start could be made by the publication of established specifications for the more important food additives, such as antimicrobial and antioxidant substances, which are of particular importance in under-developed areas.

Before toxicity tests are undertaken, the investigator must be certain that the material supplied for testing has the same specifications as that which will subsequently be used commercially. He must also know the nature and quantity of the more important impurities, since these may represent a greater health hazard than the additive itself. The chemical nature and physical properties of the substance under test may guide the investigator in the design of his experiments since they may indicate possible pathways of absorption and metabolism, as well as likely biological effects. It should be emphasized, however, that the toxic or other biological effects of the test material cannot be predicted solely from a consideration of its chemical and physical properties.

Food additives are usually substances that cause some change in the properties of food materials. While the changes that are intentionally induced in this way should be to the advantage of the consumer, it is clearly necessary to ensure that other concomitant changes are not disadvantageous. The nature of the probable reactions with food materials can often be predicted and appropriate investigations should be planned to define any significant changes that may occur. These changes may be of two types. The first is concerned with modification of nutritional value. This should be studied by direct measurement of the appropriate nutrients by suitable chemical or biological assay methods. The second is the formation of new and possibly toxic substances by the modification of food ingredients. Changes in the test substance consequent upon cooking, storage or the application of other procedures, including changes which may result from its addition to the diets of the experimental animals, must also be borne in mind. These points must be studied, if necessary, by the toxicological investigation of treated food materials. A safety factor may be introduced in this type of experiment by giving the animals food deliberately overtreated to a measured extent.

^a *Wld Hlth Org. techn. Rep. Ser.*, 1957, **129**, 13, 19 ; *FAO Nutrition Meetings Report Series*, 1957, No. 15, pp. 13, 19

If the amount of the substance added to food has to fall within specified limits, an adequate method of quantitative assessment is essential. If details of a method of isolation and analysis of the test material that is effective when applied to the finished product can be provided at the outset, this may greatly facilitate investigation and acceptance. It is sometimes overlooked that no accurate assessment of the hazard attending the use of a substance, even though it is of known toxicity, can be made unless the quantities present in food can be measured.

3. ANIMAL STUDIES

The successful use of experimental animals depends upon the care taken in their selection and maintenance throughout the experiment. It is important to have a well-designed animal house with adequate ventilation and temperature control.

As much as possible should be known about the normal duration of life and the incidence of natural disease, tumours in particular, in the animals used in long-term studies. Such information is necessary in order that the significance of any disease in the experimental groups can be assessed. For this reason rats or mice from a known strain, either bred in the laboratory or obtained from the same commercial breeder, should be used so that such information can be gradually assembled. In other species, the age and previous history of the animals should be known where possible.

For feeding tests, rats should preferably be housed singly, and given a basic diet of consistent composition adequate to support growth and reproduction under ordinary conditions. When the investigator wishes to examine toxicity under conditions of dietary deficiency, a special diet will obviously be needed. The routine use of inadequate diets in investigations of the toxicity of a food additive is, however, not recommended.

Before starting a toxicological investigation, careful consideration should be given to the experimental design to ensure that, by means of the proper statistical treatment, the maximum amount of information may be extracted from the data. Whenever measurements are made of differences between the control and experimental animals, the significance of such differences should be estimated statistically.

A major difficulty arises in connexion with the number of animals needed in order to obtain reasonable confidence limits within which the failure to detect an effect may be expressed. The application of relevant statistical principles indicates the need to use several hundred animals at each dose level in order to assure with reasonable probability that the occasional reactor—e.g., 1 in 100—may be observed in any experiment.^{1, 7} Even so it may be very difficult to detect such an abnormal reactor and

impossible to attribute the effect to the material under test. It is unrealistic to suggest the use of such large numbers. Instead, reliance can be placed on an ability to observe a response in the majority of the animals receiving dose levels far in excess of those recommended for human consumption.

3.1 Acute toxicity studies

3.1.1 *Definition*

The phrase "acute toxicity test" implies the study of the effects produced by the test material when administered in a single dose.

3.1.2 *Objectives*

The acute toxicity tests should give sufficient information to enable comparisons of the toxicity of related materials to be made and to provide the necessary information for the planning of further studies. Acute toxicity tests may indicate variation among species and yield some information on the signs of intoxication and pathological effects.

3.1.3 *Procedures*

3.1.3.1 *Species and sex.* It is advisable to employ at least three species, one of which should be a non-rodent. Both sexes should be used in at least one species.

3.1.3.2 *Conduct of experiment.*^{2, 3, 6, 7, 8, 15} When doses greater than 5 g per kg of body-weight produce no deaths in the test animals an accurate determination of the lethal dose is unnecessary. With lethal doses under 5 g/kg the LD₅₀ in one species should be determined by an appropriate method. For other species, it is desirable to determine the approximate lethal dose, where this is less than 5 g/kg, in order to indicate whether there is an important difference in species susceptibility.

3.1.3.3 *Mode of administration.* The test material should be administered orally and parenterally. Where possible it should be administered as a solution in water, edible oil or other suitable solvent; if this is not possible an inert suspending agent may be used. In all cases control data should be available on any vehicle employed.

3.1.3.4 *Observations.* The animals should be observed for a period of 2 to 4 weeks, depending on their condition. Observation should include the onset, nature and duration of toxic signs, as well as mortality. It is important that autopsies be performed on some animals that die and on some of the survivors. Microscopic examination of tissues should be carried out if the macroscopic study indicates that it is needed.

3.2 Short-term toxicity studies

3.2.1 *Definition*

The designation "short-term toxicity test" (sometimes called "sub-acute toxicity test" in the rat, and "chronic toxicity test" in the dog) implies the study of the effects produced by the test material when administered in repeated doses over a period up to 10% of the expected life-span of the animal (commonly considered to be about 90 days for the rat and one year for the dog).

3.2.2 *Objectives*

The purposes of the short-term test are to examine the biological nature of toxic effects, to assess possible cumulative action, the variation in species sensitivity, the nature of macro- and microscopic changes, and the approximate dose level at which these effects occur. It may yield information sufficient to show that the test material is too toxic to warrant further study. It may also provide guidance for the selection of dosage for long-term tests and indicate special studies that may be necessary.

3.2.3 *Procedures*

3.2.3.1 *Species and sex.* At least two species, including a rodent and a non-rodent, should be used. In most cases the rat is the animal of choice, unless it has been shown in acute toxicity experiments to be peculiarly insensitive. Among the non-rodents the dog, pig or monkey may be suitable.

3.2.3.2 *Number of animals.* With rodents the number of animals should be enough to allow for a statistical evaluation of the data. Usually, 10-20 animals of each sex on each dosage level are used; however, if some groups are to be retained for the long-term studies, the number should be sufficiently large to meet the requirements of these tests (see section 3.3.3.2, page 11). In the case of non-rodents, it is desirable to have at least 2 males and 2 females on each dosage level.

3.2.3.3 *Age of the animals.* The rats should be put on test shortly after weaning, so that observations may be made during the period of maximum growth.

3.2.3.4 *Dosage.* If little is known about the toxicity of the test material it is preferable to do a range-finding experiment before doses are selected. In the feeding experiments a sufficient number of levels should be selected to ensure that at least one level has no effect, and that doses are included which produce definite toxic effects, if this is possible. If no effects are observed at dosage levels of 10% in the diet, no useful purpose is served by employing higher levels. It is essential that a control group on the untreated diet be included in the experiment.

3.2.3.5 *Mode of administration.* In feeding studies the test material must be uniformly mixed with the diet. It may be necessary to use a volatile solvent, such as alcohol or acetone, to attain good dispersion of the test material. Care must be taken to remove the solvent prior to feeding. In studies of cumulative action, other modes of administration may be more helpful. If the test material is related to another with well-defined biological action it may be an advantage to include a group of animals receiving this substance as a positive control.

3.2.3.6 *Observations.* Observations should include general appearance, behaviour, growth and mortality. In some cases estimation of food intake, studies of blood and urine chemistry and organ function tests may be indicated. The study of the organs should include macroscopic and microscopic examination and measurement of the relative organ weights in the test and control groups. In many cases the most important organs to observe in detail are the liver and the kidney. Selection of the other organs for examination will depend upon the judgement of the investigator. Special attention should be given to the possibility of carrying out any biochemical studies that might lead to a better understanding of the effects of the material.

3.3 Long-term (chronic) toxicity studies

3.3.1 *Definition*

The designation "long-term toxicity test" implies the study of the effects produced by the test material when administered in repeated doses over a longer period of time; usually the major portion of the expected life-span of short-lived species, sometimes covering the entire life-span and more than one generation of such species.

3.3.2 *Objectives*

Long term toxicity tests are carried out to ascertain the maximum dosage level which produces no discernible ill-effects when administered over the major portion of the life-span of the experimental animal, and to reveal effects which are not predictable from short-term tests.

3.3.3 *Procedures*

3.3.3.1 *Species and sex.* In most cases the rat is the species of choice. Both sexes must be used. Under certain conditions the use of other species may be indicated.

3.3.3.2 *Number of animals.* A sufficient number of animals must be used in each experimental group to provide data for adequate statistical

analysis. Consideration should be given to the expected mortality so that a sufficient number of survivors will be available for examination at the end of the experiment. This usually means that about 25 rats of each sex must be started on each level of dietary feeding. If the experiment is designed to involve the sacrifice of animals for pathological examination while it is in progress, additional animals in each group may be necessary.

3.3.3.3 *Age of the animals.* See section 3.2.3.3, page 10.

3.3.3.4 *Dosage.* In selecting the dosage levels to be fed, considerable information can be gained from the acute and especially from the short-term toxicity tests. In many cases, two dosage levels, and a control of the basal diet without the additive, are sufficient. The lowest dose should be so selected that animals receiving it throughout the experimental period will be expected to show no discernible ill-effects. On the other hand, the highest dosage level should, if possible, be such that a definite effect is produced by the test material. It should not, however, be so high that survival is markedly decreased; dietary levels higher than 10% should not be used.

Dosage levels are commonly stated in terms of percentage by weight of the test material in the basal diet. However, dosage may be related to body weight, body surface, or caloric intake to ensure uniformity of dosage throughout the experimental period and to facilitate comparison of data among species, including man.

3.3.3.5 *Mode of administration.* See section 3.2.3.5, page 11. In testing specifically for carcinogenicity it may be necessary to consider the advisability of parenteral administration.

3.3.3.6 *Duration of experiment.* It is customary to terminate long-term experiments on rats at the end of two years, since this is usually considered to cover the major portion of their life-span. However, it may often be desirable to terminate the experiments before this time—e.g., between 12 and 18 months—in order to avoid confusing signs of toxicity with the complicated pathological changes which occur in old animals. On the other hand, in special cases—e.g., where tumour formation is of primary concern—total life-span studies, extending over two generations, in at least one species, have been advocated and may be desirable.^{9, 12, 13}

3.3.3.7 *Observations.* In addition to the observations outlined in section 3.2.3.6 (page 11), attention should be given to effects on reproduction, lactation and offspring. Furthermore, it may be desirable to carry out macroscopic and microscopic examinations periodically during the course of the long-term test.

3.4 Biochemical and other special investigations

An adequate knowledge of the metabolism and biochemical effects of a food additive has provided in some cases a satisfactory basis for recommending its use or rejection. Thus, such food additives as glycerol monostearate and monosodium glutamate have been accepted without much question because of the general knowledge of fat absorption and the amino acid composition of foods. Most of the descriptions of chronic toxicity tests that have been put forward have included some general recommendations for metabolic and biochemical studies.

The aspects of metabolic and biochemical activity that might be profitably studied include the route and rate of absorption of the test material, the levels of storage in the tissues and the subsequent fate of the stored material. Studies of the metabolism of the material, together with the identification of the metabolites, might be extended to include balance experiments in which an attempt is made to account for the administered dose as metabolites excreted or material stored in the body. These studies would be done in the first instance with high dosage levels and should be extended to include doses nearer the levels proposed in food and the study of the effects of continuous administration. Other investigations might include the examination of enzymic processes which may be affected, the effect of additives on the nutritive value of the diet, and the possibility of the formation of toxic substances during processing, storage and household preparation.

In some cases it may be desirable, in order to learn more about the mode of action, to carry out certain studies in which pharmacodynamic techniques are used. Such investigations might well reveal effects which are not apparent in the short- and long-term feeding tests, for instance, effects on the cardiovascular, autonomic, nervous or reproductive systems.

Although there is some basic information, for example, on the metabolism of phenolic compounds, the isolation and identification of the main metabolites are often difficult. Newer techniques such as chromatography and radio-active tracer methods have helped to meet the problem of separating unknown compounds from highly complex mixtures. There are examples of a major metabolite being inactive and a minor one being more active than the original material from which both were derived. Balance studies in which all the metabolites have to be accounted for are much more difficult than the usual study in which the identification of one or two metabolites is all that is accomplished. It may be desirable to carry out such balance studies on food additives but this would be a formidable undertaking.

Most metabolic studies must be done at high dosage levels in the first instance, if the experimenter is to have any chance of isolating the metabolites. Metabolic processes are influenced by the size of the dose, and the

proportion of the dose excreted as a particular metabolite may vary at different dosage levels. Once methods of isolation have been worked out, tracer techniques would enable the metabolism of small doses to be studied, but many technical problems would still be met. Most of the metabolic work has been done after a single dose or a small number of doses have been given. Few studies have been made of metabolic processes during continuous administration, although there has been some speculation on the possible effects of such dosage. In the present state of knowledge, the toxicity of many compounds cannot be explained on the basis of their metabolism.

Biochemical studies on food additives are long-term projects involving basic research and they are unlikely to replace the chronic toxicity test in the foreseeable future. Those concerned with the safety of food additives should, however, encourage fundamental research into the metabolism and biochemistry of these compounds as a basis for estimating their safety.

4. VALUE AND INTERPRETATION OF TESTS AND CONSIDERATION OF MARGINS OF SAFETY

The interpretation of the evidence involves considerations of the validity of the information provided, its applicability to man and the use of margins of safety in the estimation of the safe level of intake.

4.1 Physical and chemical characteristics

The physical and chemical nature of any material must be known before consideration can be given to its possible use as a food additive. It is necessary to decide whether the available information on the composition of the material, the assured level of purity, and the methods of isolation, analysis or assessment are adequate to ensure proper control under the conditions of use.

4.2 Acute toxicity tests

An assessment of acute toxicity is often used to give an indication of the usefulness of a food additive. Clearly a satisfactory food additive is likely to be a substance of low acute toxicity. A substance that shows appreciable acute toxicity should be regarded with suspicion until its safety under conditions of use has been demonstrated by further studies.

Frequently, owing to the low toxicity of the test material, it is only possible to state that the LD_{50} exceeds 5 g/kg. The main value of the acute tests in such cases is to provide information on the effects of the test material

on biological systems. These observations are of great importance since they may indicate the further studies needed.

Acute toxicity tests should provide information on species specificity. The objective is to reduce the possibility that some important effect that might occur in man is being overlooked in the main test animal.

4.3 Longer term studies

Short-term and long-term toxicity studies should provide data on the cumulative effects of the test material.

4.3.1 *Well-defined effects*

Data on death of the animals, convulsions or other well-defined effects can usually be expressed quantitatively and it may be possible to calculate the effective dose for 50% of the animals treated (ED_{50}). The statistical significance of such observations should be assessed.

4.3.2 *Differences in weight gain*

If a statistically significant difference in weight gain is found, it is essential that food intake should be checked. If the differences in weight gain can be fully accounted for by the differences in food intake, the result may be attributable either to anorexia or lack of palatability of the food. Paired feeding may be necessary to establish this point. Adverse effects of high dosage levels on the palatability of the experimental diet need not be relevant to the health hazard involved.

4.3.3 *Nutritional effects*

If food intake differences do not account for differences in weight gain, appropriate studies should be carried out to determine whether absorption or utilization of food materials is at fault, or some impairment of their nutritional value has taken place. The nature and extent of any significant nutritional damage should be separately defined by appropriate assay procedures.

4.3.4 *Study of different organs*

4.3.4.1 *Functional tests.* Functional tests are available for the study of the gastro-intestinal, hepatic, renal, haematopoietic, nervous and reproductive systems and can be applied to some of the common laboratory animals. Tests may be of two types, i.e., relatively simple screening tests, or more complex studies in which the different aspects of the function may be separately analysed. They provide useful quantitative information, but it must be remembered that vital organs commonly possess a considerable

reserve so that functional inadequacy may not be apparent until appreciable damage has occurred.

4.3.4.2 *Macroscopic appearance.* Careful and critical autopsy is important. The observations made cannot be readily measured quantitatively, but they serve as indices of the need for more detailed study.

4.3.4.3 *Relative organ weights.* Organ weights, related to body weight or some other common index, can be compared in experimental and control groups. Statistically significant differences indicate the need for more detailed study. Such differences, however, do not necessarily indicate, nor does their absence exclude, the existence of any deleterious effect.

4.3.4.4 *Histopathology.* Differences in microscopical appearances may be the earliest detectable sign of toxicity. Instances have been reported in which the histopathological method was the only one that revealed a deleterious effect. The importance of histopathological studies in this field is, therefore, apparent. On the other hand, it is only an extremely small proportion of the vast numbers of microscopical preparations that has provided useful positive data in the toxicological studies on food additives. Every effort should be made to reduce the load of routine histopathological examination in these studies by improving the simpler and less time-consuming observations, such as macroscopic appearance and organ weights, so that critical histopathological study may eventually be restricted to those occasions on which it will be of the greatest value.

4.3.4.5 *Organs likely to yield useful data*

(a) *Organs of elimination.* The main organs of elimination, the kidneys, liver and gastro-intestinal tract should be subjected to appropriate study.

(b) *Haematopoietic and reproductive systems.* While these tissues have an importance in their own right, they are perhaps of special interest in the investigations under discussion because they are the sites of active cell proliferation and their study may consequently assist in the elucidation of cytotoxic effects.

(c) *Other organs.* Where there is any special indication of possible effects in other organs, such as the thyroid or the brain, appropriate investigations should be carried out.

4.3.5 *Carcinogenic action*

The occurrence of any tumours should be given full attention. If more tumours are observed in the treated animals than in the controls, or if a chemical relationship between the additive under examination and known carcinogens exists, a special study directed towards the evaluation of possible carcinogenic action is indicated. Although such studies can be valuable for the detection of carcinogenicity, there are unfortunately no

completely satisfactory methods available at this time for the evaluation of this hazard for man. However, in the case of substances not clearly suspected of a carcinogenic action on the basis of present knowledge, the life-span test suggested can be considered to be a reasonable safeguard against the inclusion of a carcinogen as a food additive.

The Committee considers that the problems of chemical carcinogenesis and mutagenic action, which are not confined solely to food additives, are important enough to merit further consideration at a later date by a group, having among its membership a number of appropriately qualified workers in the field of cancer research. The Committee believes, however, that no proved carcinogen should be considered suitable for use as a food additive in any amount.

4.4 Estimation of the probable safe levels for human use

Undoubtedly, adequate evidence from human studies is the most satisfactory for the assessment of the human hazard. However, many difficulties arise in conducting such experiments and, for this reason, reliance must be placed on other forms of evidence. So far as the animal experiments are concerned, the use of high dosage levels of the test substance and the spread of the investigations over a number of different species make it reasonable to extrapolate the data to man.

From these various investigations a dosage level can be established that causes no demonstrable effect in the animals used. In the extrapolation of this figure to man, some margin of safety is desirable to allow for any species difference in susceptibility, the numerical differences between the test animals and the human population exposed to the hazard, the greater variety of complicating disease processes in the human population, the difficulty of estimating the human intake and the possibility of synergistic action among food additives.

It will be useful to try to define here the standard daily dietary dose. This is taken to be the amount of the food additive that might be expected to be consumed by an average adult eating a normal diet as determined from some appropriate dietary survey. It should be assumed in these calculations that all the foods likely to be treated with the additive will contain it at the level proposed.

It is inescapable that some arbitrary factor must be applied in order to provide an adequate margin of safety. Where the maximum ineffective dose in animals is calculated in g/kg body-weight, a margin of safety of the order of 100 has been widely used. In the absence of any evidence to the contrary, the Committee believes that this margin of safety is adequate.

The accuracy with which the maximum ineffective dose in animals can be defined clearly varies with the data that can be obtained. Where the proposed additive has a low toxicity, it may not be possible to demonstrate

any adverse biological effect. Provided that any effects which might be predicted on the basis of current knowledge have been satisfactorily excluded, the hundredfold margin of safety may also be applied to the maximum ineffective dose administered in such cases. This clearly limits the possible daily level of use of a food additive. This margin of safety covers most of the substances so far proposed as additives. However, further consideration might have to be given in specific cases involving relatively inert substances.

5. RECOMMENDATIONS TO FAO AND WHO

The Committee recommends that FAO and WHO should :

- (1) give every possible encouragement to research studies that may assist in the development of better methods for the evaluation of the safety of food additives ;
- (2) convene a Joint FAO/WHO Expert Committee on Food Additives for the purpose of drawing up agreed specifications for a number of the more important food additives ;
- (3) consider referring the problem of the possible carcinogenic and mutagenic action of food additives to an appropriate group of experts ;
- (4) explore the possibility of arranging for the exchange of unpublished information relating to investigations on the safety for use of food additives.

6. SUMMARY AND CONCLUSIONS

1. The Committee recognized the need to give guidance to workers engaged in the biological testing of food additives. The establishment of a uniform set of experimental procedures that would be standardized and obligatory would, however, raise many difficulties.
2. Scientists working in this field have a responsibility not only to indicate as comprehensively as possible the work that should be undertaken, but also to evaluate evidence upon which decisions on safe use may be made.
3. The Committee emphasizes the need for specifications of identity in chemical and physical terms as recommended in its first report and recommends that the publication of agreed specifications of the more important food additives should be undertaken.
4. An outline of the procedures for acute, short-term and long-term toxicity studies is given. These tests have been considered from the point of view of the contribution that each may make in determining safety for use.

5. An adequate knowledge of metabolic and biochemical effects of a food additive may provide, in some cases, a satisfactory background for recommendations on safety for use. Some biochemical aspects that might be profitably studied are listed.

6. The interpretation of the evidence involves consideration of the validity of the information provided, its applicability to man and the use of margins of safety in the estimation of the safe level of intake.

7. The Committee regards as indispensable the continuation and expansion of research bearing on the procedures used in assessing the safety of food additives as the only means of providing simpler, more exact, and more economical methods.

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