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**TOXICOLOGICAL EVALUATION OF  
CERTAIN FOOD ADDITIVES WITH A  
REVIEW OF GENERAL PRINCIPLES  
AND OF SPECIFICATIONS**

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

Geneva, 25 June – 4 July 1973



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TOXICOLOGICAL EVALUATION OF CERTAIN FOOD  
ADDITIVES  
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Expert Committee on Food Additives

CORRIGENDA

Page 21, paragraph 5.5.2, line 4

*delete the sentence* The former conditional ADI . . . body weight.  
*insert* The former unconditional ADI — 0-50 mg/kg body weight  
— was retained.

Page 36, *under* Emulsifiers, lines 1, 4, and 8

*delete* footnote reference

Page 37, *under* Thickening agents (contd), line 1

*delete* acetylated distarch phosphate  
*insert* acetylated distarch phosphate

Page 37, section D

*against* alginic acid and its ammonium, calcium, potassium, and sodium salts

*delete* 0-25

*insert* 0-50

Page 37, *under* Miscellaneous food additives, line 1

*delete* calcium acetate, chloride and sulfate Not limited

*insert* calcium acetate, chloride, gluconate, and sulfate Not limited<sup>35</sup>

Page 37, *under* Miscellaneous food additives, line 10

*against* 1,2-propylene glycol

*delete* 0-125

*insert* 0-25

Page 38, footnote 23

*delete* As sum of total ... tartaric acids,

Page 38, *after* footnote 34

*insert* <sup>35</sup> The intake of calcium gluconate should comply with the ADI for gluconic acid.

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Geneva, 25 June - 4 July 1973

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# **TOXICOLOGICAL EVALUATION OF CERTAIN FOOD ADDITIVES WITH A REVIEW OF GENERAL PRINCIPLES AND OF SPECIFICATIONS**

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

A Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 25 June to 4 July 1973. The meeting was opened by Dr B. Dieterich, Director, Division of Environmental Health, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization. Dr Dieterich, in his opening address, briefly reviewed the origins and work of the Committee as well as WHO programmes in related areas, particularly those on environmental health monitoring and criteria.

### **1. INTRODUCTION**

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955,<sup>1</sup> sixteen meetings of the Joint FAO/WHO Expert Committee on Food Additives have been held (see Annex 1). The present meeting was convened on the recommendations made in the sixteenth report of the Committee. Its terms of reference were : (1) to review the principles for evaluating the safety of food additives ; (2) to make a toxicological re-evaluation of the food additives listed in Annex 2 ; (3) to review the specifications of the substances listed in Annex 2 ; (4) to discuss an approach to the evaluation of flavouring substances.

### **2. GENERAL CONSIDERATIONS**

#### **2.1 Comments on the agenda**

##### *2.1.1 Substances added to the agenda*

The agenda was originally drafted partly in terms of compound groupings having similar technological functions. In considering individual

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<sup>1</sup> *FAO Nutrition Meetings Report Series*, 1956, No 11 ; *Wld Hlth Org. techn. Rep. Ser.*, 1956, No 107.

substances in the different groups, the Committee decided to review the toxicological evaluation of a number of related substances that did not fall within the specific functional classifications, i.e. certain phosphates, calcium chloride, oxystearin, polyvinyl pyrrolidone, propylene glycol and sorbitol. The monographs on the toxicological re-evaluation of lactic, citric, glutamic and tartaric acids also include the re-evaluation of their salts with certain bases accepted for food use. A review of the specifications for the substances listed above will be carried out by a future Committee.

#### 2.1.2 *Substances deleted from the agenda*

As a number of important studies on saccharin were in progress, the Committee decided not to deal with this substance for the time being.

### 2.2 Principles governing the use of food additives

The first meeting of the Joint FAO/WHO Expert Committee on Food Additives was held in Rome in December 1956 and dealt with general principles governing the use of food additives. It was recommended that food additives should be used only after authorization by the appropriate authorities and that their legal control should be based on the system of permitted or positive lists. Two factors have to be taken into account in evaluating a substance proposed for use as a food additive, namely technological efficacy and safety in use. The criteria for both must be satisfied before a substance can be formally accepted as a food additive.

The use of a food additive should be technologically justified on the basis of advantage to the community and the consumer, and, as stated in the Committee's sixth report,<sup>1</sup> the level of use should not exceed the lowest level that is effective in good manufacturing practice.

The most important factor for the acceptance of a substance as a food additive is the establishment of its safety in use. This principle was clearly stressed in the Committee's first report.<sup>2</sup> It implies that an adequate toxicological evaluation has to be made (see section 2.4).

Specifications for food additives are required as a safeguard against the presence of harmful contaminants and also to ensure that food additives are adequately defined (see section 2.3), since otherwise their technological usefulness and toxicological properties cannot be assessed in a pertinent manner.

In view of public concern about the use of food additives and, in particular, the number of additives that may be permitted for use in any one food, the Committee drew attention to the many safeguards that exist in this

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<sup>1</sup> See Annex 1, ref. 6.

<sup>2</sup> See Annex 1, ref. 1.



domain. Legislation provides safeguards against adulteration and debasement in the quality or nutritive value of food. More specifically, there are safeguards concerning the acceptability of individual food additives and of combinations of food additives.

The Committee has frequently reiterated the general principles laid down in its first report concerning the safe use of food additives. However, it should be pointed out that the absolute safety of the foods themselves cannot be guaranteed since natural components of normal foods may exhibit toxic properties. By contrast, the scrutiny to which food additives are subjected in arriving at a toxicological evaluation makes for their safety in practice. The potential hazard, if any, that the use of a food additive may present to the consumer must be weighed against the benefits that it confers; for example, a food additive may provide protection against the development in food of microbial toxins or mycotoxins.

When several additives are used in a food, the most important factor to be considered is the level of exposure of the consumer to each food additive rather than the total number used. In fact, the use of a number of food additives to achieve a technological effect, each at a lower level than would be needed if it were used alone, may provide an additional measure of safety, as long as there are no undesirable interactions between them. Such interactions may be chemical or biological in nature. The Committee considered possible chemical interactions between food additives that might yield toxic substances, thus precluding the simultaneous use of the additives in the same food. As regards biological interactions, the Committee acknowledged the theoretical possibility of some subtle potentiation of toxicity, but in practice such an effect has rarely been encountered. Compounds that are closely related in chemical structure or function may exhibit additive and, more rarely, synergistic biological effects. In several instances the Committee has allocated ADIs to groups of such compounds, thus providing a safeguard in the event of a number of such food additives being used in a single food.

In the years that have elapsed since the publication of its first report, the Committee has repeatedly stressed the importance of the general principles concerning the safe use of food additives. It emphasized once again that, provided these principles are adhered to, the goal of the safety in use of food additives is attainable in practice.

### **2.3 Principles governing specifications**

General principles governing specifications were summarized in the tenth report<sup>1</sup> in which the Committee also recommended a review of specifications in the light of current information regarding the production,

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<sup>1</sup> See Annex 1, ref. 13.

use, composition, and purity of food additives and methods of analysis. The work of the Committee has, however, been continuously expanding to cover not only simple chemicals but also complex substances such as enzyme preparations and natural flavouring materials. For certain food additives derived from plants or animals, it is necessary to establish microbiological criteria. This is the case, for example, with some natural food colours, vegetable gums, gelatin, carrageenan, pectin, modified starches, and enzymes. The Committee felt that there was a need to review the general principles with regard to their applicability to these substances and to consider the need for additional criteria.

## 2.4 Principles governing toxicological evaluation

Principles of evaluation and procedures for the toxicological testing of food additives were dealt with in the second,<sup>1</sup> fifth,<sup>2</sup> sixth,<sup>3</sup> and tenth<sup>4</sup> reports of the Committee. A WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives<sup>5</sup> reviewed the advances made in the various relevant disciplines and made recommendations on principles for toxicological evaluation.

Over the years, the Committee has extended its scope to include the evaluation not only of intentional food additives but also of certain antibiotics, trace elements, contaminants, and processing aids. Consequently, there have been some minor changes in its approach to the problem of toxicological evaluation, especially with respect to the allocation of "acceptable" intakes.

There are two stages in the toxicological evaluation of a substance proposed for use as a food additive. The first is the collection of relevant data, which are usually derived from experimental testing in laboratory animals and, whenever possible, from observations in man. The second is the interpretation and assessment of the data in order to arrive at a decision about the acceptability or otherwise of the substance as a food additive.

### 2.4.1 Testing procedures

The procedures for testing food additives are summarized in Annex 3, on the basis of previous reports of Joint FAO/WHO Expert Committees. This summary has been provided only because some early reports are no longer available and no attempt has been made to review the procedures critically. It must be stressed that the summary is intended only to indicate

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<sup>1</sup> See Annex 1, ref. 2.

<sup>2</sup> See Annex 1, ref. 5.

<sup>3</sup> See Annex 1, ref. 6.

<sup>4</sup> See Annex 1, ref. 13.

<sup>5</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

general guidelines. The procedures should be modified as necessary, depending on the nature of the substance to be tested, and should take advantage of recent developments in toxicological techniques.

#### 2.4.2 *Interpretation of findings*

The objective in assessing the toxicological data on food additives is to ensure their safety for the consumer on the basis of all the evidence available to the Committee at the time. Future results with present methods or with techniques yet to be developed will necessitate reassessments that may lead to changes in earlier decisions.

The general procedure adopted has been to establish an acceptable daily intake (ADI) for man for each food additive, or for groups of additives. When the toxicological data are derived from animal experiments, their extrapolation to man involves the application of a safety factor to the highest "no-effect level" obtained in animal studies.

#### 2.4.3 *No-effect level*

When applied to data from animal experiments, the term "no-effect level" refers to the level of a substance that can be included in the diet of a group of animals without toxic effects. With certain substances, the highest level that can be incorporated in a diet fails to produce any effect. However, some food additives do exert toxic effects when fed at high levels and for these the maximum no-effect level is used. The maximum no-effect level should be determined in the most appropriate animal species and be based on the most pertinent criteria of toxicity.

At its meetings, the Committee has considered a variety of effects that, in the present state of knowledge, are not deemed to be of toxicological significance, provided they are fully attributable to normal physiological adjustment and are reversible. They include, for example, changes in intestinal flora, laxative effects due to bulk or osmotic load, caecal enlargement and diminished growth rate caused by high levels of non-digestible substances, and liver hypertrophy and induction of microsomal enzymes due to gross overloading with certain metabolizable substances.

#### 2.4.4 *Extrapolation of data to man*

In the extrapolation of animal data to man, the application of a safety factor is required for the following reasons : to allow for any differences in sensitivity between the animal species and man ; to allow for wide variations in sensitivity among the human population ; to allow for the fact that the number of animals tested is small compared with the size of the human population that may be exposed.

It was recognized by the Committee that the expression of the ADI in terms of body weight (BW) does not reflect the relative exposure of animals of different size as accurately as would the metabolic mass, which is equal to  $W_b^{0.75}$ . However, in practice the method of expressing the dose in terms of mg/kg body weight has proved satisfactory.

A safety factor of 100, the figure recommended by the Committee in its second report,<sup>1</sup> has been widely accepted. But it would be unreasonable to apply this figure too rigidly, for example, in the case of substances that are normal constituents of the human diet or are normal intermediary metabolites. When there are adequate data to show that, in the human body, a substance is converted by digestion or metabolism to a normal constituent of the diet, or that a substance is not absorbed from the gastrointestinal tract, these data are used in the evaluation. When toxicological data derived from experiments in man are available, they may be used to provide a lower safety factor since they obviate the need for interspecies extrapolation.

On the other hand, there may be reasons to increase the safety factor—for example, when the amount and/or the quality of toxicological information are limited. Furthermore, the nature of the toxic effect produced by an additive at very high levels might demand an increase in the factor in order to ensure safety in use.

#### 2.4.5 *Acceptable daily intake for man*

The acceptable daily intake (ADI) for man, expressed on a body weight basis, is the amount of a food additive that can be taken daily in the diet, even over a lifetime, without risk.

An ADI is allocated only to substances for which the available data include either the results of adequate short-term and long-term toxicological investigations or satisfactory information on the biochemistry and metabolic fate of the compound, or both.

An ADI may be allocated temporarily, pending the provision of additional data within a stated period of time. This measure implies that the toxicological data are adequate to ensure the safety in use of the additive during the time for which the temporary ADI applies. If the additional data requested do not become available within the stated period, the temporary ADI may be withdrawn at a future meeting of the Committee.

An ADI without an explicit indication of the upper limit of intake (“not limited”) may be assigned to substances of very low toxicity, especially those that are food constituents or that may be considered as

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<sup>1</sup> See Annex 1, ref. 2.

foods or normal metabolites in man. An additive having a "not limited" ADI must meet the criteria of good manufacturing practice—for example, it should have proven technological efficacy and be used at the minimum level of efficacy, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance.

There may be circumstances in which the ADI is not applicable. Thus it may be exceeded for special dietary purposes—for example, in the case of modified celluloses, to reduce the energy content of the diet. On the other hand, the ADI for glutamic acid and glutamates does not extend to foods for infants under three months old.

In previous reports a conditional ADI was allocated to a number of substances, often in addition to an unconditional ADI. Variations in the rules for applying conditional ADIs have given rise to some confusion. For this reason, the Committee considers that the allocation of conditional ADIs should be abandoned.

ADIs are intended as guides only and may be exceeded, after consultation with experts, in circumstances in which there may be important advantages in doing so. In the opinion of the Committee, an ADI provides a sufficiently large safety margin to ensure that there need be no undue concern about occasionally exceeding it provided the average intake over longer periods of time does not exceed it.

#### *2.4.6 The allocation of ADIs to additives that are related chemically and toxicologically*

As a number of food additives are closely related chemically and toxicologically, the Committee adopted in its seventh and later reports a system of grouping additives for purposes of evaluation—for example, phenolic antioxidants, emulsifiers of the polyglycerol ester series, the polyoxyethylene sorbitan esters, modified celluloses and propylene glycol derivatives. In such cases, the ADI level is expected to cover all specified members of the group that may be included in the diet. A given food additive may be related to two groups, in which case the level in the diet must not exceed the maximum acceptable level for either group. The problem is not so complicated as it may appear at first sight, since many of the substances in a group of additives have technologically related functions and are therefore likely to be used as alternatives to each other.

#### *2.4.7 Exclusion from the ADI of amounts occurring naturally in foods*

The ADI includes only the amounts of the substance used as an additive, excluding "amounts naturally present in food". There is a single exception, namely the ADI for phosphates, which includes all phosphates, whatever the source.

#### 2.4.8 Other conclusions

Other conclusions on food additives may be summed up in the following terms :

(a) " Decision postponed " (pending clarification of matters related to technological use) ;

(b) " No ADI allocated " (in the absence of sufficient information to establish safety, or of adequate specifications) ;

(c) " Not to be used " (where there is sufficient information on which to base such a decision).

#### 2.5 Tissue uptake and storage of macromolecular food additives

Information on the tissue storage of macromolecular materials is frequently lacking. In the case of propylene glycol alginate, labelled with  $^{14}\text{C}$  either in the alginate or propylene glycol moiety, whole-body autoradiography of treated mice served to establish lack of absorption of the alginate.<sup>1</sup> Unlike lipid-soluble substances whose storage within the body is non-specific, some macromolecular materials are localized within lysosomes of cells of the reticuloendothelial system. Degraded carrageenan behaves in this way and is retained at the sites of storage, for example in Kupffer cells of rhesus monkeys, for six months or longer after administration has ceased.<sup>2</sup> The consequences of uptake and storage of macromolecular substances in reticuloendothelial cells are not well understood, but there is some indication that alterations of phagocytic function may occur.

In view of the availability of a range of techniques (analytical, histochemical, ultrastructural, and autoradiographic) to supplement light-microscopic observations, further studies to obtain information on the tissue storage of macromolecular food additives should be carried out.

#### 2.6 Allergenicity of food additives

A number of food additives are known to cause allergic manifestations in susceptible individuals. Sometimes these are due to occupational exposure and, while this aspect is outside the terms of reference of the Committee, the evidence obtained in such instances is used in assessing allergenicity. No approval would be given for the use as a food additive of a substance causing serious or widespread hypersensitivity reactions. For food additives with an allergenic potential, the inclusion on food labels of lists of the individual additives used has been suggested as a

<sup>1</sup> Sharratt, M. & Dearn, P. (1971) Unpublished report submitted by BIBRA.

<sup>2</sup> Abraham, R., Goldberg, L. & Coulston, F. (1972) *Exp. Mol. Pathol.*, **17**, 77 ; Warren, S. & Sommers, S. C. (1954) *J. Amer. Med. Ass.*, **154**, 189.

means of enabling sensitive individuals and their physicians to identify the possible sources of allergic reactions. The practicability and effectiveness of such a measure is uncertain and the matter requires further consideration.

### 3. PRINCIPLES GOVERNING THE ASSESSMENT OF FLAVOURING SUBSTANCES

In its eleventh report,<sup>1</sup> the Committee evaluated some flavouring substances and suggested criteria to be used in ascertaining which groups should be given priority for further study. The present Committee reconsidered the question of the safety of flavouring substances in the light of the approach adopted by the Council of Europe.<sup>2</sup> It also took cognizance of the various efforts being made to establish the average and maximum use and intake levels of flavourings in some countries.

#### 3.1 Toxicological evaluation

In general, the Committee considered that the procedures adopted by the Council of Europe represent a useful and practical approach; the problem of evaluating flavouring substances is one that cannot be solved simply by adopting the processes traditionally used by the Committee to evaluate other types of food additive. While concurring with the concept put forward by the Council of Europe, the Committee wishes to draw attention to the following issues:

(a) In listing flavouring substances it is difficult to take into account the many indigenous materials, notably herbs, that are peculiar to specific regions. The Council of Europe lists are not comprehensive and are unlikely to incorporate all the flavouring substances actually used.

(b) In the opinion of the Committee, the guide to the testing and toxicological evaluation of flavouring substances provided by the Council of Europe goes too far beyond the enunciation of principles, entering into detailed protocols that are not only unnecessary but also tend to create a rigid set of testing requirements. The Committee cannot emphasize too strongly the need to maintain flexibility at all times in the approach to toxicity testing since each compound presents an individual and unique problem.

#### 3.2 Specifications

For the preparation of chemical specifications, flavouring products may be categorized as follows:

<sup>1</sup> See Annex 1, ref. 14.

<sup>2</sup> Council of Europe, *Natural and artificial flavouring substances* (unpublished documents).

- (a) Extracts (preparations) of plants or animal materials ;
- (b) Essential oils of plant origin ;
- (c) Individual chemical compounds obtained from natural sources or produced synthetically.

In accordance with general principles for the acceptance of chemicals for food use, it is necessary to identify and define the pure substance in each case. For the categories listed above, however, the following considerations are of importance :

(a) Preparations manufactured by extraction, steam distillation, maceration, or similar means are complex mixtures. For food use, these mixtures should be prepared by safe and unobjectionable processes involving the use of food-grade extraction or carrier solvents. Trade specifications have already been prepared for the former commercial products, and suitable specifications for the latter, i.e., for food use, should be prepared in the near future. The requirement that these preparations should be manufactured by the use of food-grade solvents, or by steam distillation or some other innocuous operation, is considered adequate. The source of raw materials as well as limits for possible contaminants must be specified.

(b) The processes used in the manufacture of essential oils may be similar to those described above, but essential oils almost always contain a major constituent that can be identified and defined by chemical or physical means. Trade specifications are available for such products and it should be possible to examine them and develop suitable specifications for their use in food. Limits may be necessary for constituents of possible toxicological significance.

(c) For individual chemical substances, whether natural or synthetic, it should be possible to prepare suitable specifications in the usual form. The principles set out in the eleventh report<sup>1</sup> would apply in such cases.

#### 3.2.1 *Methods for the analysis of flavours in food*

The Committee recognized the problems involved in developing suitable analytical procedures, particularly for flavouring materials present in the final food ready for consumption. The methods so far developed cannot discriminate between flavouring substances present naturally in food and the same substances added during processing.

### 4. REVIEW OF SPECIFICATIONS

The Committee reviewed the specifications prepared at previous meetings for antimicrobials, antioxidants, anticaking agents, emulsifiers, gums, modified starches, and certain other food additives. Some specific recom-

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<sup>1</sup> See Annex 1, ref. 14.



mendations and general observations were made. A revision of the nomenclature is required in certain instances, and the chemical composition of many substances needs to be described more precisely.

Since many of the specifications were prepared at different meetings, there are some discrepancies in the definitions or descriptions of the substances. The specifications thus need editing to bring them into line as regards technical terms and general presentation. Furthermore, a certain number of specifications need updating in the light of present chemical, technological, and toxicological knowledge.

There are inconsistencies in the manner in which limits on heavy metal contaminants, arsenic, and fluorine have been applied to closely related additives. While particular manufacturing processes may occasionally demand the application of different limits, there is a need to bring most of the specifications into line in accordance with the principles given in the tenth report.<sup>1</sup> In some instances it should be possible to reduce the limit of impurities and encourage good manufacturing practice.

Attention was also drawn to the possible contamination of fatty acid derivatives with chlorinated dioxin ("chick oedema factor"). More information is needed on the incidence of such contamination and methods for its detection. There is also a likelihood that polyoxyethylene-containing esters may be contaminated by ethylene glycol, diethylene glycol, and ethylene chlorhydrine during manufacture. More information is required on the extent and level of such contamination.

The new and better assay methods that have become available in recent years must be taken into consideration and incorporated into specifications or general methods where appropriate. The general methods of analysis were fully reviewed. The inclusion of thin-layer and gas-liquid chromatographic procedures and of more advanced methods for the determination of arsenic (colorimetric procedure) and various heavy metals (atomic absorption procedures) was specifically recommended. Some of the obsolete and inaccurate methods for identification and purity tests need to be replaced. The listing of test solutions should be brought up to date.

Some assay methods for certain food additives call for reference standard samples. The monographs should indicate the sources of supply of such substances, and measures should be taken to ensure that they are made available.

The Committee recommended that all specifications should be revised in the light of the above review and published in a single compendium for easy reference. This should also include details of relevant analytical methods, test solutions, and reagents.

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<sup>1</sup> See Annex 1, ref. 13.

## 5. COMMENTS ON FOOD ADDITIVES ON THE AGENDA

The Committee re-evaluated, in the light of new data, a number of food additives that had undergone toxicological evaluation at previous meetings and for which chemical specifications had been prepared. For many of these substances no change in the acceptance was required and no comment will be made on them. For others, the level of the ADI was either increased or, in a few cases, decreased, for the reasons set out below. The results of the evaluations are summarized in Annex 4 and details of the substances are given in the monographs.<sup>1</sup>

### 5.1 Anticaking agents

#### *Calcium, potassium, and sodium ferrocyanide*

The metabolic data available for humans suggest that ferrocyanides are reabsorbed by the renal tubules. A full examination in man of the pharmacokinetic behaviour and biotransformation of orally ingested ferrocyanides is required. On the basis of the no-effect level in the rat, the ADI for man was estimated at 0-0.025 mg/kg body weight; a large safety factor was applied pending the results of the further work mentioned above.

#### *Others*

Other anticaking agents considered were: salts of myristic, palmitic, and stearic acids (ammonium, calcium, magnesium, potassium, sodium), silicon dioxide and certain silicates (aluminium, calcium, sodium aluminosilicate) to which a 'not limited' acceptance was given, and magnesium silicate (talc and magnesium trisilicate) to which a temporary 'not limited' acceptance was given pending further studies.

### 5.2 Antimicrobials

#### *Benzoic acid*

The ADI for this substance, estimated on the basis of the no-effect level in the rat, remains unchanged at 0-5 mg/kg body weight, the conditional ADI being deleted.

#### *Diethylpyrocarbonate*

Urethan (ethyl carbamate) is known to be formed in beverages treated with diethylpyrocarbonate. Urethan is a wide-spectrum carcinogen, producing tumours in many organs and in all species tested, and acts

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<sup>1</sup> See p. 2.

across the placental barrier. Since there was uncertainty about the significance of the presence of urethan at low levels, the previous acceptance of diethylpyrocarbonate was revoked.

*Ethyl, methyl and propyl p-hydroxybenzoates*

On the basis of adequate long-term studies in rats, the ADI in man was increased to 0–10 mg/kg body weight (as the sum of ethyl, methyl, and propyl esters).

*Hexamethylenetetramine*

The toxicological effects of this substance appear to be due to the liberation of formaldehyde and formic acid, the metabolism of which are known. Long-term studies in rodents show a no-effect level of 500 mg/kg. However, there was a report of fetotoxicity in the dog when the substance was administered at a level of 31 mg/kg; at 15 mg/kg there was no effect. The ADI was accordingly lowered to 0–0.15 mg/kg. The Committee noted the *in vitro* demonstration of the chemical interaction of hexamethylenetetramine with nitrite to form nitrosamine. Although the experimental conditions were considerably more severe than those occurring in food processing, it was thought inadvisable to use hexamethylenetetramine when there is a possibility that nitrite might also be present in the food.

*Nitrate and nitrite, potassium, and sodium salts*

The toxic effects of nitrates are due to their transformation to nitrites, in food or water or in the gastrointestinal tract in young infants, whose capacity for secreting acid in the stomach is lower than that of adults. High levels of nitrates occur naturally in a number of vegetables and these may create problems in infants less than three months of age. The Committee discussed the possibility of nitrosamine formation resulting from the use of nitrates and nitrites as food additives and emphasized the need for further work on this problem. Cognizance was taken of recent studies showing that ascorbic acid can prevent the nitrosation of oxytetracycline to diemethyl-nitrosamine *in vitro*<sup>1</sup> and the possible prevention of carcinogenesis by nitrosamines formed *in vivo*. It was acknowledged that in the absence of suitable alternatives, nitrates and nitrites are required to control toxin-forming microorganisms such as *Clostridium botulinum*. For nitrates, the previously estimated ADI (0–5 mg/kg) was retained and the conditional ADI deleted. For nitrites the previous ADI was lowered to 0.2 mg/kg body weight.

*Sodium diacetate*

The previous conditional ADI was deleted.

<sup>1</sup> Mirvish, S. S., Wallcave, L., Eagen, M. & Shubik, P. (1972) *Science*, **177**, 65.

#### *Sorbic acid and its calcium and potassium salts*

The ADI for man was set at 0–25 mg/kg body weight, a figure that includes the figures previously given for the conditional ADI.

#### *Sulfur dioxide, sodium and potassium metabisulfite, sodium sulfite and sodium hydrogen sulfite*

Recent long-term and 3-generation studies on rats, using metabisulfite in a diet with added thiamine, provided a higher no-effect level than that indicated by the earlier studies in which metabisulfite was administered in drinking water. The ADI could thus be increased.

#### *Others*

Other antimicrobials considered were: acetic acid and its potassium and sodium salts, hydrogen peroxide, copper and cupric sulfate, and formic acid.

### **5.3 Antioxidants and synergists**

#### *Ascorbic acid and potassium and sodium ascorbate*

Ascorbic acid exhibits no toxicity after the administration of relatively large doses in animals or man. The estimated ADI for man was accordingly raised to 0–15 mg/kg body weight over and above the amount of ascorbic acid naturally present in foods.

#### *Ascorbyl palmitate and stearate*

On the basis of adequate long-term studies in the rat, the ADI for man was estimated at 0–1.25 mg/kg body weight (as the palmitate or stearate, or the sum of both).

#### *Butylated hydroxyanisole, butylated hydroxytoluene, and dodecyl gallate, octyl, and propyl*

Recent evidence indicates possible effects on reproduction in the rat, when butylated hydroxyanisole, alone or with propyl gallate, is mixed with lard in the diet. The ADIs of the compounds listed above were therefore made temporary, pending further studies on reproduction using butylated hydroxyanisole alone or in mixtures.

#### *Ethylenediamine tetraacetate, disodium, and calcium disodium salts*

On the basis of the no-effect level in the rat, the ADI for man was estimated at 0–2.5 mg/kg body weight, which includes the previous unconditional and conditional ADI figures.

#### *Guaiac resin*

The Committee decided to use the alternative name of guaiac resin, instead of guaiac gum for this substance, in order to distinguish it more

easily from the polysaccharide gums, which have a different chemical structure and a different use. A specification for the substance was prepared and will be published in due course. On the basis of the no-effect level in the rat, the ADI for man was estimated at 0–2.5 mg/kg body weight.

*Isoascorbic acid and sodium salt*

Adequate short and long-term studies have been carried out in the rat. The biochemical studies indicate that isoascorbic acid is readily metabolized and does not affect the urinary excretion of ascorbic acid. On the basis of the no-effect level in the rat, the ADI for man was estimated at 0–5 mg/kg body weight.

*Isopropyl citrate mixture and monoisopropyl citrate*

On the basis of the no-effect level in the rat, the ADI for man was estimated at 0–14 mg/kg body weight.

*Stearyl citrate*

The data for this compound were carefully re-evaluated and the previous ADI increased.

*Thiopropionic acid and dilauryl ester*

The previous conditional ADI was deleted. Because studies on the distearyl ester were insufficient for evaluation purposes, the ADI does not include this compound.

*α-tocopherol and mixed tocopherols concentrate*

On the basis of clinical experience with this vitamin, the ADI for man was estimated at 0–2 mg/kg body weight, which includes the previous unconditional and conditional figures.

*Others*

Other antioxidants considered include citric acid and its salts and nordihydroguaiaretic acid.

#### **5.4 Emulsifiers and stabilizers**

*Ammonium salts of phosphatidic acids*

Tentative specifications prepared at the thirteenth meeting of the Committee were changed into final specifications. An ADI was established.

*Diacetyl tartaric and fatty acid esters of glycerol*

For these substances, the previous unconditional and conditional ADIs were converted to an ADI of 0–50 mg/kg on the basis of the results of biochemical and metabolic studies and feeding tests in animals.

*Esters of glycerol and thermally oxidized soybean fatty acid*

The Committee was informed that the so-called esters of glycerol and thermally oxidized soybean fatty acid were in fact simple mixtures of thermally oxidized soybean oil and mono- and diglycerides and not true esters. It was not considered necessary to have a specification for such mixtures, since the Committee had prepared separate specifications for the two substances involved. It was accordingly decided to withdraw the specification prepared at the fifteenth meeting of the Committee. The tentative specification for thermally oxidized soybean oil had been prepared at the same meeting.

In the absence of adequate information relating these compounds to compounds that had been studied toxicologically, the Committee was unable to establish an ADI.

*Fatty acid esters of glycerol with acetic, citric, lactic and tartaric acids*

These substances were treated as a group and given a collective "not limited" ADI except for tartaric acid, the ADI of which should not exceed 30 mg/kg body weight.

*Lecithin*

In view of biochemical and nutritional experience with lecithin, the Committee changed the ADI to "not limited".

*Mono- and diglycerides*

The Committee considered that mono- and diglycerides differed little from food so that their use need not be limited.

*Polyglycerol esters of fatty acids*

The Committee examined metabolic data showing that the fatty acid moiety of this group of compounds is metabolized by the body in the same way as normal fatty acids and the polyol portion is rapidly excreted via the kidney. In the light of these data, together with the findings from extensive studies on animals and some data from man, the former conditional ADI was converted to an ADI of 0-25 mg/kg body weight.

*Polyglycerol esters of interesterified ricinoleic acid*

On the basis of no-effect levels determined in long-term animal-feeding studies, the ADI was established at 0-7.5 mg/kg body weight.

*Polyoxyethylene (20) sorbitan esters of lauric, stearic, palmitic, and oleic acids and triesters of stearic acid*

The Committee reviewed this series of compounds and decided to retain the former unconditional ADI as the ADI.

*Polyoxyethylene (8) stearate and polyoxyethylene (40) stearate*

The Committee decided to group these substances on the ground that they were broken down and treated by the body in a similar manner. An ADI was therefore established for the two compounds used in combination.

*Propylene glycol esters of fatty acids*

There is evidence that the propylene glycol esters of fatty acids are hydrolysed to propylene glycol and fatty acids. The Committee's evaluation was based on the content of propylene glycol, for which an ADI had been established.

*Sorbitan esters of palmitic and stearic acids and triesters of stearic acid*

The Committee considered that the partial esters of sorbitan have been thoroughly investigated in both short-term and long-term experiments, as well as in man. The data was considered as covering sorbitan mono- and tristearates and sorbitan monopalmitate, and an ADI was assigned to the whole group.

*Others*

Other emulsifiers considered include: cholic and desoxycholic acid; hydroxylated lecithin; mixed tartaric, acetic, and fatty acid esters of glycerol; sucrose esters of fatty acids; and sucroglycerides.

## 5.5 Thickening agents

### 5.5.1 Celluloses

The Committee allocated a group ADI of 0-25 mg/kg body weight to hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methyl cellulose, methyl ethyl cellulose, and sodium carboxymethyl cellulose. A "not limited" acceptance was given to microcrystalline cellulose.

### 5.5.2 Others

*Alginic acid and its ammonium, calcium, sodium, and potassium salts*

Alginic acid and its four salts were considered together. Additional data were available to show that the alginates *per se* are poorly absorbed. The former conditional ADI — 0-50 mg/kg body weight — was replaced by an ADI of 0-25 mg/kg body weight.

*Carrageenan and furcellaran*

When the Committee first considered carrageenan, it was unaware either of the numerous varieties coming under this general heading or of the possible toxicological implications of difference in sources, methods of isolation and preparation, molecular forms, and molecular-weight fractions.

That these factors cannot be ignored is suggested by reports of the ulcerogenic and other effects of carrageenans in various animal species. The precise nature of the carrageenan tested was, however, not always clearly stated. One unequivocal conclusion that may be drawn from the animal work is that a particular preparation of degraded carrageenan (used as a therapeutic agent and not as a food additive) is ulcerogenic under certain circumstances. There is no definite evidence that any of the various forms of undegraded carrageenan are capable of eliciting pathological changes in experimental animals or man. At present, there is little information on the proportions of degraded carrageenans occurring in the various food additive forms. Also, there is a paucity of data on the extent to which carrageenans are degraded during the processing of acid foods containing them as additives. It is imperative to establish the exact significance of such degradation from a toxicological standpoint.

In the present state of knowledge it is therefore concluded that forms of carrageenan of low molecular weight should be avoided in food. These considerations also affect specification and analysis, questions that are dealt with in the monograph on carrageenan.<sup>1</sup> Similarly, the evaluation of food-grade carrageenan given in this report takes into account the new biological data available. The specifications will need suitable amendments in the light of additional information, e.g. on viscosity and on the dispersity of molecular weights.

#### *Pectins*

The specification for pectin prepared by the Committee at its thirteenth meeting was reviewed. A revised tentative specification covering both the nonamidated and amidated pectins was prepared in the light of additional information now available. The specification will be published in due course. A separate ADI was established for amidated pectin.

The Committee considered also agar, arabic gum, and guar gum, for each of which a "not limited" acceptance was given. For carob bean gum, karaya gum, and tragacanth gum, no evaluation was possible with the data provided.

#### *5.5.3 Modified starches*

On the basis of the recommendations contained in the thirteenth report,<sup>2</sup> and in view of the fact that both general and individual specifications for different enzyme preparations intended for food processing have since been prepared, the Committee decided that only these enzyme preparations should be used for the manufacture of enzyme-treated starches. It was

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<sup>1</sup> See p. 2.

<sup>2</sup> See Annex 1, ref. 19.



also decided to prescribe the same limits of impurities for enzyme-treated starches as for chemically treated starches.

The Committee finalized tentative specifications for hydroxypropyl distarch phosphate that had been prepared at the thirteenth meeting. The opportunity was taken of reviewing the specifications for other chemically treated starches and carrying out some minor amendments in the light of more detailed information now available. A test procedure for the determination of residual manganese in bleached starch was provided. With the exception of hydroxypropyl distarch phosphate and starch sodium succinate, all the modified starches had already been given a "not limited" ADI.

## 5.6 Miscellaneous

A miscellaneous group of compounds unrelated functionally or structurally was considered by the Committee.

### *L-glutamic acid and its monosodium, potassium, ammonium, and calcium salts*

The Committee considered glutamic acid together with its sodium, potassium, ammonium, and calcium salts. Although findings concerning the susceptibility of neonates to glutamates were considered controversial, the Committee concluded it would be prudent not to apply the ADI to infants under 12 weeks of age. Data suggesting that ingestion by mothers of high levels of glutamates had harmful effects on offspring (exposed *in utero* or when suckling) were considered less convincing, and the former ADI of 0-120 mg/kg body weight, calculated as glutamic acid, was retained.

### *Lactic acid and its sodium, potassium, and calcium salts ; propionic acid and its sodium, potassium, and calcium salts*

Since lactic and propionic acids are normal constituents of food and normal intermediary metabolites in man, it was considered unnecessary to set ADI limits for them. There is, however, some evidence that the neonate has difficulties in utilizing the *D*(-)-isomer of lactic acid; it was considered, therefore, that neither this nor the racemate should be used in foods for infants less than three months old. Metabolic studies on the utilization of *D*(-)-lactic acid in infants are needed.

### *Phosphoric acid, polyphosphates, and their sodium, potassium, calcium and magnesium salts, calcium and magnesium phosphates tribasic*

Ingested phosphates from natural sources must be considered together with those from food additive sources, this being a departure from the usual practice in estimating ADIs. The evaluation was based on the normal range of dietary intake and the no-effect level in the rat, to which a safety factor was applied. The acceptable total dietary phosphorus intake for

man was estimated at 0-70 mg/kg body weight. This figure applies to the sum of added phosphate and food phosphate and to diets that are nutritionally adequate with respect to calcium. However, in the case of a high intake of calcium, proportionally higher amounts of phosphate would be acceptable and vice versa.

While reviewing the specifications for phosphates and polyphosphates, the Committee's attention was drawn to the necessity for tests for cyclic phosphate, which may occur in polyphosphates and create certain health hazards. It was decided to prescribe a limit for cyclic phosphates in the specifications for polyphosphates.

#### *Polyvinyl pyrrolidone*

It was noted that, in the past, the Committee had expressed certain reservations about the dependability of the available data on the body storage of polyvinyl pyrrolidone, particularly in the intestinal lymph nodes. Because of more general concern about the effects of stored macromolecules, the Committee decided to discontinue the previous conditional ADI and not to set an ADI for this substance.

#### *1,2-propylene glycol*

The Committee took note of the metabolic behaviour of the compound as well as toxicological data, and an ADI of 0-25 mg/kg body weight was set.

#### *L(+)-tartaric acid, potassium, sodium, and mixed potassium-sodium salts*

The long-term study in rats showed no adverse effects at the highest level tested, and the substances have been used medicinally for long periods. The evaluation was based on the experimental data, the metabolic inertness of tartrates and the fact that they are normal constituents of food. The ADI for man was estimated at 0-30 mg/kg body weight (calculated as L(+)-tartaric acid).

In the miscellaneous group, the Committee also considered calcium acetate, calcium chloride, calcium sulfate, food-grade mineral oil, oxytearin, and sorbitol.

## 6. FUTURE WORK

1. The Codex Alimentarius Commission, the principal organ of the Joint FAO/WHO Food Standards Programme, has the function of drawing up international food standards to protect the health of the consumer and facilitate international trade in food. To provide the necessary scientific basis for the Codex Food Standards, the Committee should continue to evaluate those food additives on which it is proposed to include provisions in the standards.

2. In 1970, recognizing the potential hazards of food additives to the consumer, the Twenty-third World Health Assembly adopted a resolution<sup>1</sup> requesting Member States to transmit to WHO any decision to limit or prohibit the use of a food additive. WHO was requested to transmit this information to other Member States and where desirable, to arrange for the toxicity of the food additive to be evaluated by the Joint FAO/WHO Expert Committee on Food Additives.

In addition, it is considered advisable that, before finalizing the agenda of any future meetings of the Committee, FAO and WHO should request the members of the WHO Expert Advisory Panel on Food Additives and the FAO panel of experts to collect new toxicity and chemical data on food additives and to solicit suggestions for the Committee's discussions.

3. Future meetings of the Committee should continue to give consideration to the technological efficacy of food additives, including their mechanism of action. This will help Member governments and the appropriate Codex Committees to define good manufacturing practices and facilitate decisions on the acceptance of additives for food use.

4. The general principles governing the establishment of specifications should be reviewed in the light of the current work and future programme of the Committee. In addition, the review of all the specifications should be completed as soon as possible, and suitably revised or edited specifications, together with the required methods of analysis, should be issued in a single compendium.

5. The methods and procedures for the toxicity testing of food additives should be comprehensively reviewed and brought into line with advances in toxicology and cognate disciplines.

6. Further information is needed on the chemical fate of food additives in foods and their interaction with food components, with particular reference to the formation of toxic substances that would need to be taken into account in toxicological evaluations.

7. Recent studies demonstrate that the capacity to absorb and store certain macromolecular materials within the body extends to some substances used as food additives. It is recommended that similar studies should be conducted on the full range of additives added to food in macromolecular form.

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<sup>1</sup> World Health Organization (1973) *Handbook of resolutions and decisions of the World Health Assembly and the Executive Board, Vol. 1, 1948-1972*, Geneva, p. 156 (WHA23.50).

## 7. RECOMMENDATIONS

1. Meetings of the Expert Committee should be held regularly by FAO and WHO in order to deal with the topics enumerated in section 6.
2. WHO should systematize the means at its disposal for the identification of food additives that new data have shown to be of dubious safety.
3. In considering the toxicity of food additives, consideration has been given to a number of natural constituents of food that have deleterious effects. WHO, in collaboration with FAO, should convene a meeting of experts to assess the health hazards presented by these substances.

### ACKNOWLEDGEMENT

The Committee records its appreciation of the helpful information provided by Dr P. Shubik, Director of the Eppley Institute for Research in Cancer, College of Medicine, University of Nebraska, Omaha, Nebr., USA.

## Annex 1

### REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. General Principles Governing the Use of Food Additives. First Report. *FAO Nutrition Meetings Report Series*, 1957, No. 15 ; *Wld Hlth Org. techn. Rep. Ser.*, 1957, No. 129.
2. Procedures for the Testing of Intentional Food Additives to Establish their Safety for Use. Second Report. *FAO Nutrition Meetings Report Series*, 1958, No. 17 ; *Wld Hlth Org. techn. Rep. Ser.*, 1958, No. 144.
3. Specifications for Identity and Purity of Food Additives (Antimicrobial Preservatives and Antioxidants). Third Report. These specifications were subsequently revised and published as *Specifications for Identity and Purity of Food Additives*, vol. I. *Antimicrobial Preservatives and Antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962.
4. Specifications for Identity and Purity of Food Additives (Food Colours). Fourth Report. These specifications were subsequently revised and published as *Specifications for Identity and Purity of Food Additives*, vol. II. *Food Colours*, Rome, Food and Agriculture Organization of the United Nations, 1963.
5. Evaluation of the Carcinogenic Hazards of Food Additives. Fifth Report. *FAO Nutrition Meetings Report Series*, 1961, No. 29 ; *Wld Hlth Org. techn. Rep. Ser.*, 1961, No. 220.
6. Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants. Sixth Report. *FAO Nutrition Meetings Report Series*, 1962, No. 31 ; *Wld Hlth Org. techn. Rep. Ser.*, 1962, No. 228.
7. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Emulsifiers, Stabilizers, Bleaching and Maturing Agents. Seventh Report. *FAO Nutrition Meetings Report Series*, 1964, No. 35 ; *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 281.
8. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Food Colours and Some Antimicrobials and Antioxidants. Eighth Report. *FAO Nutrition Meetings Report Series*, 1965, No. 38 ; *Wld Hlth Org. techn. Rep. Ser.*, 1965, No. 309.
- \*9. Specifications for Identity and Purity and Toxicological Evaluation of Some Antimicrobials and Antioxidants. *FAO Nutrition Meetings Report Series*, 1965, No. 38A ; WHO/Food Add/24.65.
- \*10. Specifications for Identity and Purity and Toxicological Evaluation of Food Colours. *FAO Nutrition Meetings Report Series*, 1966, No. 38B ; WHO/Food Add/66.25.
11. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-Treatment Agents, Acids and Bases. Ninth Report. *FAO Nutrition Meetings Report Series*, 1966, No. 40 ; *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 339.

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\* These documents can be obtained on request from : Food Additives, World Health Organization, 1211 Geneva 27, Switzerland, or Food Policy and Food Science Service, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy.

12. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Emulsifiers and Stabilizers and Certain Other Substances. Tenth Report. *FAO Nutrition Meetings Report Series*, 1967, No. 43 ; *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 373.
- \*13. Toxicological Evaluation of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-treatment Agents, Acids and Bases. *FAO Nutrition Meetings Report Series*, No. 40A, B, C ; WHO/Food Add/67.29.
14. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Flavouring Substances and Non-Nutritive Sweetening Agents. Eleventh Report. *FAO Nutrition Meetings Report Series*, 1968, No. 44 ; *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 383.
- \*15. Toxicological Evaluation of Some Flavouring Substances and Non-Nutritive Sweetening Agents. *FAO Nutrition Meetings Report Series*, 1968, No. 44A ; WHO/Food Add/68.33.
- \*16. Specifications and Criteria for Identity and Purity of Some Flavouring Substances and Non-Nutritive Sweetening Agents. *FAO Nutrition Meetings Report Series*, 1969, No. 44B ; WHO/Food Add/69.31.
17. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Antibiotics. Twelfth Report. *FAO Nutrition Meetings Report Series*, 1969, No. 45 ; *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 430.
- \*18. Specifications for the Identity and Purity of Some Antibiotics. *FAO Nutrition Meetings Report Series*, 1969, No. 45A ; WHO/Food Add/69.34.
19. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Food Colours, Emulsifiers, Stabilizers, Anticaking Agents, and Certain Other Substances. Thirteenth Report. *FAO Nutrition Meetings Report Series*, 1970, No. 46 ; *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445.
- \*20. Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anticaking Agents and Certain Other Substances. *FAO Nutrition Meetings Report Series*, No. 46A ; WHO/Food Add/70.36.
- \*21. Specifications for the Identity and Purity of Some Food Colours, Emulsifiers, Stabilizers, Anticaking Agents and Certain Other Food Additives. *FAO Nutrition Meetings Report Series*, No. 46B ; WHO/Food Add/70.37.
22. Evaluation of Food Additives. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation : Some Extraction Solvents and Certain Other Substances ; and a Review of the Technological Efficacy of Some Antimicrobial Agents. Fourteenth Report. *FAO Nutrition Meetings Report Series*, 1971, No. 48 ; *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 462.
- \*23. Toxicological Evaluation of Some Extraction Solvents and Certain Other Substances. *FAO Nutrition Meetings Report Series*, 1971, No. 48A ; WHO/Food Add/70.39.
- \*24. Specifications for the Identity and Purity of Some Extraction Solvents and Certain Other Substances. *FAO Nutrition Meetings Report Series*, 1971, No. 48B ; WHO/Food Add/70.40.
- \*25. A Review of the Technological Efficacy of Some Antimicrobial Agents. *FAO Nutrition Meetings Report Series*, 1971, No. 48C ; WHO/Food Add/70.41.
26. Evaluation of Food Additives. Some Enzymes, Modified Starches, and Certain Other Substances : Toxicological Evaluations and Specifications and a Review of the Technological Efficacy of Some Antioxidants. Fifteenth Report. *FAO Nutrition Meetings Report Series*, 1972, No. 50 ; *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 488.

27. Toxicological Evaluation of Some Enzymes, Modified Starches and Certain Other Substances. *FAO Nutrition Meetings Report Series*, 1972, No. 50A; *WHO Food Additives Series*, 1972, No. 1.
28. Specifications for the Identity and Purity of Some Enzymes and Certain Other Substances. *FAO Nutrition Meetings Report Series*, 1972, No. 50B; *WHO Food Additives Series*, 1972, No. 2.
29. A Review of the Technological Efficacy of Some Antioxidants and Synergists. *FAO Nutrition Meetings Report Series*, 1972, No. 50C; *WHO Food Additives Series*, 1972, No. 3.
30. Evaluation of Certain Food Additives and the Contaminants Mercury, Lead and Cadmium. Sixteenth Report. *FAO Nutrition Meetings Report Series*, 1972, No. 51; *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 505.
31. Evaluation of Mercury, Lead, Cadmium and the Food Additives Amaranth, Diethylpyrocarbonate and Octyl Gallate. *FAO Nutrition Meetings Report Series*, 1972, No. 51A; *WHO Food Additives Series*, 1972, No. 4.

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## Annex 2

### LIST OF SUBSTANCES ON THE AGENDA

1. Antimicrobials
2. Antioxidants
3. Anticaking agents
4. Emulsifiers
5. Gums
6. Modified starches
7. Miscellaneous
  - (a) Mineral oils
  - (b) Monosodium glutamate
  - (c) Saccharin

## PROCEDURES FOR TESTING FOOD ADDITIVES

As no single pattern of tests could adequately cover a range of substances so diverse in structure and function as food additives, it would be unreasonable to impose a rigidly uniform set of experimental procedures. It cannot be emphasized too strongly that the conduct of experiments designed to test the safe use of food additives is the responsibility of the specialized scientist. For these reasons, it is possible only to formulate general guidelines on testing procedures.

### Acute toxicity studies

The following statements from the second report of the Joint FAO/WHO Expert Committee on Food Additives<sup>1</sup> remain valid :

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

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. 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 LD<sub>50</sub> 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.

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.

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.

Studies on acute toxicity in several animal species should make it possible to gain an idea of the apparent mode of action of the chemical, e.g., whether it acts as an anticholinesterase, central nervous system depressant or convulsant, a metabolic stimulant, or a liver- or kidney-damaging agent.

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<sup>1</sup> See Annex 1, ref. 2, p. 9.



Comparison with well known chemicals may be useful when a new chemical falls into the same general category.

#### **Biochemical studies**

A number of types of study are included under this heading. Significant biochemical aspects include mode, rate and degree of absorption, levels of storage in organs and tissues, metabolic transformation, and mode and rate of elimination. Modifications of substances during metabolism may significantly affect their toxicity. Knowledge whether or not a food additive is rapidly metabolized into innocuous degradation products, rapidly excreted or accumulated in certain organs or tissues may be of great value in assessing potential hazards.

It is highly important that information about the metabolism and distribution of a substance undergoing testing should be obtained at an early stage since it may then be possible to make similar investigations in man. The information from such investigations will make it possible to choose, for further experimentation, the animal species corresponding most closely to man in the absorption and metabolism of the substance and thus to obtain data on animal toxicity that will enhanced predictive value.

In biochemical studies, the possibility of reactions of the additive with food constituents should be taken into account. Such reactions are of two types. First, the nutritional value of the food may be affected ; this possibility may be studied by chemical or biological assay methods. Second, new and possibly toxic substances may be formed ; these must be investigated by the usual toxicological procedures. Cooking, storage, or the application of other technological procedures may also alter the test substance. It may be necessary to undertake a toxicological investigation of treated food materials ; here a margin of safety may be introduced by conducting the tests with food that has been deliberately overtreated to a measured extent.

Some additives may be converted into substances already present naturally in food in much greater amounts. If the biochemical evidence shows that the sole effect of the additive is to make a small contribution to existing metabolic loads from food components, there is no need for detailed toxicological studies, a case in point being that of the lactic and fatty acid esters of glycerol, which are completely hydrolysed in the intestinal lumen with the formation of substances already present in the diet in much greater quantities.

If a series of chemical analogues can be shown to give rise to the same main metabolic product and to other compounds that are already present in the organism in greater quantities, or that can be readily and safely metabolized, it may be sufficient to carry out toxicological studies on a suitable representative of the series.

The effects of the additive on important enzyme systems in blood or tissues should be studied. For example, an increase in the level of certain marker enzymes in the serum, such as transaminases and other intracellular enzymes, may be indicative of tissue damage. Another aspect to be investigated is the induction of microsomal enzyme systems, especially in the liver. The relevance to man of these biochemical changes needs careful assessment.

#### **Short-term studies**

In the second report of the Committee,<sup>1</sup> short-term studies are discussed in the following terms :

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.

At least two species, including a rodent and a non-rodent, should be used. The number of animals should be enough to allow for a statistical evaluation of the data. 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.

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.

These recommendations are still applicable except when the available data show that in the rat (considered in the report as the animal of choice) the metabolism of the chemical being tested is not comparable with its metabolism in man.

It may be feasible or necessary to administer test substances by gavage or by capsule, e.g. in cases of limited solubility, unpleasant taste, or need for accurate dosing. These procedures may produce effects different from those produced by adding a substance to the diet, either by increasing or decreasing the rate of absorption from the gastrointestinal tract or by influencing the metabolic pathway in the bacterial flora of the upper intestine. The validity of these forms of administration will depend on the substance tested.

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<sup>1</sup> See Annex 1, ref. 2, pp. 10-11.

### Long-term toxicity

Where it is considered necessary to carry out long-term toxicity and carcinogenicity studies, the following principles formulated by a WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives<sup>1</sup> should be followed:

Scientific judgement is necessary in determining the duration of animal studies for the evaluation of an individual food additive. Where adequate biochemical and toxicological data on closely related chemicals are available, the objective becomes the detection of any deviation from the established pattern. This can usually be determined by intensive studies of a few months' duration when these are adequately designed and evaluated. Appropriate studies in humans add significantly to the adequacy of the data.

In the absence of such definitive data, or if there are reasons to suspect carcinogenic potential, longer-term studies must still be relied upon for reassurance. Recent advances in the quality of research animals, and particularly in the control of pathogens, have increased the life-span of some strains of animals. In spite of this, feeding studies adequately designed and evaluated extending up to eighteen months in mice and two years in rats are still considered adequate to ensure a minimum safeguard in evaluating the carcinogenic potential of a chemical additive. In special cases it may be desirable to prolong the observations in these species.

If there are good reasons to doubt the relevance to man of the data obtained in rodent species—for example, if the metabolism of the additive in man is found to be significantly different from that in rodents—it may be desirable to carry out investigations of longer duration in other species. In addition, the investigation of some potential toxic effects, particularly carcinogenicity, requires careful prolonged observation of the offspring. Detailed study of general appearance and behaviour, biochemical effects, metabolism and histopathology should be included and fully reported, both qualitatively and quantitatively.

### Special studies

#### *Reproduction, embryotoxicity, and teratogenicity studies*

During the preceding tests, or in a pilot experiment, careful attention should be paid to any changes likely to affect reproduction. If there is any evidence to suggest that reproduction may be affected, specific studies should be undertaken.

Reproduction studies must be carried out in a suitable species over at least two generations and may have to be continued over three or more. They should be designed to provide relevant information on fertility, progress of pregnancy, post-partum condition, and progress of mothers and offspring.<sup>2</sup>

Reproduction studies may be designed to include investigations on embryotoxicity and teratogenicity. Alternatively it may be more convenient to conduct separate investigations on these aspects. Many suggested procedures for these studies have been published, but no one particular design has yet emerged as being universally acceptable.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348, pp. 13-14.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 364.

### *Carcinogenicity and mutagenicity*

Special consideration should be given to testing for the possible carcinogenicity and mutagenic potential of substances to be used in food. The Committee noted that these matters had been dealt with by a WHO Scientific Group on the Principles for the Testing and Evaluation of Drugs for Carcinogenicity,<sup>1</sup> a WHO Scientific Group on the Evaluation and Testing of Drugs for Mutagenicity,<sup>2</sup> and the Canadian Department of Health and Welfare.<sup>3</sup> It was also noted that a Scientific Group on the Assessment of the Carcinogenicity and Mutagenicity of Chemicals will be convened by WHO in 1973.

Certain aspects need particularly careful evaluation. If, for example, a promoting effect is detected in the course of a carcinogenicity test it cannot be ignored. However, routine testing for promoting effects should not be used until more is known about its mechanism and how broadly it can be applied. Likewise, even when cancer is produced by chemicals administered by other than the oral route, their evaluation for food additive use requires experiments using oral administration.

Tissue culture methods for the study of neoplastic transformation, using chemical carcinogens, have not yet been fully developed, and it would be premature to introduce these methods into toxicity studies of food additives.

A procedure at present under investigation in routine carcinogenesis studies includes the use of two parent and offspring generations to take into account the transplacental transport of carcinogens and their transfer to milk. No recommendation can yet be made as to the suitability of this method for the toxicological investigation of food additives.

### **Observations in man**

Observations in man are of prime importance because of the differences between one species and another in reactions to toxic substances and the subsequent uncertainty when extrapolating data from animal experiments to human beings.

Studies in man may be carried out by the careful observation of individuals who have ingested the test compound. Additional data may be obtained by studying individuals exposed to additives occupationally or through accidental ingestion. Finally, studies may also be carried out in populations consuming a given additive at high levels because of ethnic proclivities of for therapeutic purposes.

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<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1969, No. 462.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 482.

<sup>3</sup> Canada, Department of Health and Welfare (1973) *The testing of chemicals for carcinogenicity, mutagenicity, and teratogenicity*, Ottawa.

Annex 4

ACCEPTABLE DAILY INTAKES \*

| <i>Substances</i>  | <i>Acceptable daily<br/>intake for man<br/>(mg/kg body weight)</i> |
|--|--|
| <b>Anticaking agents</b>   |  |
| calcium, potassium, sodium ferrocyanide  | 0-0.025 <sup>1</sup>   |
| salt of myristic, palmitic, and stearic acids (aluminum, ammonium, calcium, magnesium, potassium, sodium)                | Not limited  |
| silicon dioxide and certain silicates (aluminium, calcium, sodium aluminosilicate)                                       | Not limited  |
| magnesium silicate (talc and magnesium trisilicate)  | Not limited <sup>1</sup>   |
| <b>Antimicrobials</b>  |  |
| acetic acid and its potassium and sodium salts   | Not limited  |
| benzoic acid and its potassium and sodium salts <sup>10</sup>  | 0-5  |
| diethyl pyrocarbonate <sup>4</sup>   | Not to be used   |
| formic acid  | 0-3  |
| cupric sulfate   | No ADI <sup>5</sup>  |
| hexamethylenetetramine   | 0-0.15   |
| hydrogen peroxide  | No ADI <sup>6</sup>  |
| p-hydroxybenzoate, butyl   | No ADI allocated <sup>2</sup>                                      |
| p-hydroxybenzoate, ethyl <sup>3</sup>  | 0-10   |
| p-hydroxybenzoate, methyl <sup>3</sup>   | 0-10   |
| p-hydroxybenzoate, propyl <sup>3</sup>   | 0-10   |
| nitrate, potassium and sodium salts <sup>8</sup>   | 0-5  |
| nitrite, potassium and sodium salts <sup>9</sup>   | 0-0.2 <sup>1</sup>   |
| propionic acid and its calcium, potassium, and sodium salts  | Not limited  |
| sodium diacetate   | 0-15   |
| sorbic acid and its calcium, potassium and sodium salts <sup>11</sup>  | 0-25   |
| sulfur dioxide and sulfites (sodium and potassium metabisulfites, sodium sulfite, sodium hydrogen sulfite) <sup>12</sup> | 0-0.7  |
| <b>Antioxidants and synergists</b>   |  |
| ascorbic acid and its potassium and sodium salts   | 0-15 <sup>13</sup>   |
| ascorbyl palmitate and stearate <sup>14</sup>  | 0-1.25   |
| butylated hydroxyanisole   | 0-0.5 <sup>15</sup>  |
| butylated hydroxytoluene   | 0-0.5 <sup>15</sup>  |
| citric acid and its calcium, potassium, and sodium salts   | Not limited  |
| ethylenediaminetetraacetate, disodium and calcium disodium salts <sup>16</sup>   | 0-2.5  |
| gallates, dodecyl, octyl, propyl <sup>17</sup>   | 0-0.2  |
| guaiac resin   | 0-2.5  |

\* For footnotes, see p. 37.

| <i>Substances</i>  | <i>Acceptable daily<br/>intake for man<br/>(mg/kg body weight)</i> |
|--|--|
| <b>Antioxidants (contd)</b>  |  |
| isoascorbic acid and its sodium salt   | 0-5  |
| isopropyl citrate mixture and monoisopropyl citrate <sup>18</sup>  | 0-14   |
| nordihydroguaiaretic acid  | No ADI allocated <sup>2</sup>                                      |
| stearyl citrate  | 0-50   |
| thiodipropionic acid and dilauryl ester <sup>21</sup>  | 0-3  |
| $\alpha$ -tocopherol and mixed tocopherols concentrate <sup>22</sup>   | 0-2  |
| <b>Emulsifiers</b>   |  |
| acetic and fatty acid esters of glycerol <sup>23</sup>   | Not limited  |
| ammonium salts of phosphatidic acid  | 0-15 <sup>1</sup>  |
| cholic and desoxycholic acid and their salts   | 0-1.25   |
| citric and fatty acid esters of glycerol <sup>23</sup>   | Not limited  |
| diacetyl tartaric and fatty acid esters of glycerol  | 0-50   |
| esters of glycerol and thermally oxidized soybean fatty acid   | Decision postponed <sup>25</sup>                                   |
| hydroxylated lecithin  | No ADI allocated <sup>2</sup>                                      |
| lactic and fatty acid esters of glycerol <sup>23</sup>   | Not limited  |
| lecithin   | Not limited  |
| mixed tartaric, acetic and fatty acid esters of glycerol <sup>23</sup>   | Not limited  |
| mono- and diglycerides   | Not limited  |
| polyglycerol esters of fatty acids   | 0-25 <sup>26</sup>   |
| polyglycerol esters of interesterified ricinoleic acid   | 0-7.5  |
| polyoxyethylene (20) sorbitan esters of lauric, stearic, palmitic<br>and oleic acids and triesters of stearic acid <sup>27</sup> | 0-25   |
| polyoxyethylene (8) and polyoxyethylene (40) stearate <sup>28</sup>  | 0-25   |
| propylene glycol alginate <sup>34</sup>  | 0-25   |
| propylene glycol esters of fatty acids <sup>29</sup>   | 0-25   |
| sorbitan esters of palmitic and stearic acids and triesters of<br>stearic acid <sup>30</sup>                                     | 0-25   |
| sucrose esters of fatty acids and sucroglycerides  | 0-2.5 <sup>1</sup>   |
| <b>Thickening agents</b>   |  |
| <b>A. Celluloses :</b>   |  |
| hydroxypropyl cellulose <sup>31</sup>  | 0-25   |
| hydroxypropyl methyl cellulose <sup>31</sup>   | 0-25   |
| methyl cellulose <sup>31</sup>   | 0-25   |
| methylethyl cellulose <sup>31</sup>  | 0-25   |
| microcrystalline cellulose   | Not limited  |
| sodium carboxymethyl cellulose <sup>31</sup>   | 0-25   |
| <b>B. Vegetable gums :</b>   |  |
| arabic gum   | Not limited  |
| carob bean gum   | No ADI allocated <sup>2</sup>                                      |
| guar gum   | Not limited  |
| karaya gum   | No ADI allocated <sup>2</sup>                                      |
| tragacanth gum   | No ADI allocated <sup>2</sup>                                      |
| <b>C. Modified starches and dextrans :</b>   |  |
| acetylated distarch adipate  | Not limited  |
| acetylated distarch glycerol   | Not limited  |

| <i>Substances</i>   | <i>Acceptable daily<br/>intake for man<br/>(mg/kg body weight)</i> |
|---|--|
| <b>Thickening agents (contd)</b>  |  |
| acetylated distarch phosphate   | Not limited  |
| acid-treated starches   | Not limited  |
| alkali-treated starches   | Not limited  |
| amylose and amylopectin   | Not limited  |
| bleached starches   | Not limited  |
| distarch glycerol   | Not limited  |
| distarch phosphate  | Not limited  |
| enzyme-treated starches   | Not limited  |
| hydroxypropyl distarch glycerol   | Not limited  |
| hydroxypropyl distarch phosphate  | No ADI allocated <sup>2</sup>                                      |
| hydroxypropyl starch  | Not limited  |
| mono-starch phosphate   | Not limited  |
| oxidized starches   | Not limited <sup>1</sup>   |
| phosphated distarch phosphate   | Not limited  |
| starch acetate  | Not limited  |
| starch sodium succinate   | No ADI allocated <sup>2</sup>                                      |
| white and yellow dextrans   | Not limited  |
| <b>D. Others :</b>  |  |
| agar  | Not limited  |
| alginic acid and its ammonium, calcium, potassium, and sodium salts <sup>24</sup>     | 0-25   |
| carrageenan and furcellaran   | 0-75   |
| pectin (non-amidated)   | Not limited  |
| pectin (amidated)   | 0-25 <sup>1</sup>  |
| <b>Miscellaneous food additives</b>   |  |
| calcium acetate, choride and sulfate  | Not limited  |
| food-grade mineral oil  | Not limited  |
| L-glutamic acid, its ammonium, calcium, monosodium, and potassium salts <sup>32</sup> | 0-120  |
| lactic acid and its ammonium, calcium, potassium, and sodium salts                    | Not limited <sup>7</sup>   |
| oxystearin  | 0-25   |
| phosphoric acid and its salts   | 0-70 <sup>19</sup>   |
| polyvinyl pyrrolidone   | No ADI allocated <sup>33</sup>                                     |
| 1,2-propylene glycol  | 0-125  |
| sorbitol  | Not limited  |
| stearoyl lactic acid, calcium and sodium salts  | 0-20   |
| tartaric acid (and its potassium, sodium, and potassium-sodium salts) <sup>20</sup>   | 0-30   |

<sup>1</sup> Temporary acceptance.

<sup>2</sup> Evaluation not possible with data provided.

<sup>3</sup> As sum of ethyl, methyl, and propyl esters of *p*-hydroxybenzoic acid.

<sup>4</sup> The treatment level allocated in previous reports is withdrawn.

<sup>5</sup> Maximum acceptable daily load = 0.5 mg/kg body weight expressed as Cu.

<sup>6</sup> This substance is to be used only as an emergency measure when better methods of milk preservation are not available.

- <sup>7</sup> Neither D(-)-lactic acid nor (DL)-lactic acid should be used in infant foods.
- <sup>8</sup> As sodium nitrate.
- <sup>9</sup> As sodium nitrite.
- <sup>10</sup> As sum of benzoic acid and sodium and potassium benzoate (expressed as benzoic acid).
- <sup>11</sup> As sum of sorbic acid and calcium, potassium, and sodium sorbates (expressed as sorbic acid).
- <sup>12</sup> As SO<sub>2</sub>.
- <sup>13</sup> This figure is in addition to the ascorbic acid present naturally in foods.
- <sup>14</sup> As ascorbyl stearate or ascorbyl palmitate, or the sum of both.
- <sup>15</sup> As BHA, BHT, or the sum of both. Temporary acceptance.
- <sup>16</sup> As CaNa<sub>2</sub>EDTA (no excess of Na<sub>2</sub>EDTA should remain in foods).
- <sup>17</sup> As sum of dodecyl, octyl, and propyl gallate. Temporary acceptance. (n-Octyl gallate should not be used in beverages.)
- <sup>18</sup> As monoisopropyl citrate.
- <sup>19</sup> Total dietary phosphorus load for man. Attention should be paid to the reverse relationship with calcium intake.
- <sup>20</sup> As L(+)-tartaric acid.
- <sup>21</sup> As thiodipropionic acid.
- <sup>22</sup> As  $\alpha$ -tocopherol.
- <sup>23</sup> As sum of total glycerol esters of fatty acids and acetic, citric, lactic, and tartaric acids, provided that the total food additive intake of tartaric acid does not exceed 30 mg/kg.
- <sup>24</sup> As alginic acid.
- <sup>25</sup> Previously allocated temporary ADI is withdrawn. See section 5.4.
- <sup>26</sup> As polyglycerol esters of palmitic acid.
- <sup>27</sup> As total polyoxyethylene (20) sorbitan esters.
- <sup>28</sup> As total of polyoxyethylene (8) and (40) stearates.
- <sup>29</sup> As propylene glycol.
- <sup>30</sup> As total sorbitan esters.
- <sup>31</sup> As the sum of total modified celluloses. The ADI may be exceeded for dietetic purposes, i.e., when use is primarily intended to take advantage of the non-caloric properties of these additives.
- <sup>32</sup> As glutamic acid, additional to glutamic acid intake from all non-additive dietary sources. ADI not applicable to infants under 12 weeks of age.
- <sup>33</sup> Evaluation not possible with data provided. Previously allocated conditional ADI is withdrawn.
- <sup>34</sup> The contribution from propylene glycol alginate to total dietary propylene glycol intake from all sources should be included in the ADI for propylene glycol.



## Annex 5

### FURTHER TOXICOLOGICAL STUDIES AND INFORMATION REQUIRED OR DESIRABLE

#### 1. Anticaking agents

*Calcium, potassium, sodium ferrocyanide.* Metabolic studies in man.<sup>1</sup> If these reveal any untoward effects, a long-term study in one species will be required.

*Magnesium silicate.* Studies to elucidate the reported kidney damage in dogs by magnesium silicate. Long-term studies on talc demonstrated to be free from asbestos-like particles. A satisfactory method for estimating asbestos-like particles in talc and magnesium silicate.<sup>2</sup>

#### 2. Antimicrobials

*p-hydroxybenzoate, ethyl.* Further biochemical studies in man and animals.

*p-hydroxybenzoate, methyl.* Additional studies in man.

*Nitrites, potassium and sodium salts.* Full evaluation when results of investigations in progress become available.

*Sulfur dioxide and sulfites.* Additional mutagenic studies in a mammalian system. Studies over longer periods in man.

#### 3. Antioxidants

*Butylated hydroxyanisole.* Studies of the effect on reproduction of mixtures of BHA, BHT, and propyl gallate, and of BHA alone.<sup>2</sup>

*Butylated hydroxytoluene.* Studies of the effect on reproduction of mixtures of BHT, BHA, and propyl gallate.<sup>2</sup>

*Gallates, dodecyl, octyl, propyl.* Studies of the effect on reproduction of mixtures of BHA, BHT, and propyl gallate.<sup>2</sup>

*Stearyl citrate.* Short-term studies in two species (one a non-rodent mammalian species); some of these studies should use combinations with hard fats. Emphasis should be placed on the evaluation of kidney function.

*Thiodipropionic acid and dilauryl ester.* Biochemical studies and observations on human subjects. Specifications for the distearyl ester.

#### 4. Emulsifiers

*Ammonium salts of phosphatidic acids.* Submission of the results of the long-term study in the rat and metabolic studies in several species.<sup>1</sup> Studies on the metabolic fate of these compounds in man.

*Polyglycerol esters of fatty acids.* Properly conducted biochemical studies on other members of this group that do not conform to the specifications already established, particularly those containing short chain fatty acids.

*Stearoyl lactic acid, calcium and sodium salts.* Studies confirming that this compound is metabolized by man in the same way as by other species.

<sup>1</sup> Required by June 1974.

<sup>2</sup> Required by June 1976.

*Sucrose esters of fatty acids and sucroglycerides.* Metabolic studies on representative individual sucrose esters. Two-year studies on another sucrose ester in a non-rodent mammalian species.<sup>2</sup>

#### 5. Thickening agents

*Arabic gum.* Studies of the metabolic fate in man as well as more adequate studies in animals. Studies on the allergenic reactions reported following ingestion in man.

*Guar gum.* Results on the short-term study which is in progress to check the reliability of the older studies.

*Oxidized starches.* Results of histopathological studies.<sup>1</sup>

*Pectin (amidated).* Provision of full specifications.<sup>1</sup>

#### 6. Miscellaneous food additives

*Food-grade mineral oil.* Further work on the elucidation of the significance of stored mineral oil in the body.

*L-glutamic acid, its ammonium, calcium, monosodium, and potassium salts.* Oral non-adverse effect levels of glutamates in neonatal animals. Age correlations between neonatal experimental animals and the human infant. (Required if use is to be extended to infant foods.)

*Lactic acid and its salts.* Metabolic studies on the utilization of D(-) and DL-lactic acids in infants.

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<sup>1</sup> Required by June 1974.

<sup>2</sup> Required by June 1976.