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

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 488

FAO NUTRITION MEETINGS REPORT SERIES
No. 50

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
of the Joint FAO/WHO Expert Committee
on Food Additives

Rome, 16–24 June 1971

Published by
FAO and WHO

WORLD HEALTH ORGANIZATION
GENEVA
1972

Specifications for the substances considered in this report, monographs containing summaries of relevant biological data and toxicological evaluations, and a review of the technological efficacy of some antioxidants will be issued separately by FAO and WHO under the titles :

1. *Toxicological evaluation of some enzymes, modified starches and certain other substances*

   FAO Nutrition Meetings Report Series, No. 50 A

   WHO Food Additives Series, No. 1

2. *Specifications for the identity and purity of some enzymes and certain other substances*

   FAO Nutrition Meetings Report Series, No. 50 B

   WHO Food Additives Series, No. 2

3. *A review of the technological efficacy of some antioxidants*

   FAO Nutrition Meetings Report Series, No. 50 C

   WHO Food Additives Series, No. 3

PRINTED IN SWITZERLAND

CONTENTS

Page

1. Introduction 5

2. The problem of additives in baby foods 6

3. Principles governing chemical specifications 7

4. Principles governing toxicological evaluations 8

5. Comments on substances on the agenda 9
   5.1 Enzymes 9
   5.2 Modified starches 13
   5.3 Miscellaneous food additives 15

6. Review of the technological efficacy of some antioxidants 20

7. Recommendations 21

Annex 1. Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives 22

Annex 2. List of substances on the agenda 25

Annex 3. Report of an FAO/WHO Meeting on Additives in Baby Food 27

Annex 4. Evaluation of commercial enzymes 38

Annex 5. Evaluation of modified starches 40

Annex 6. Evaluation of miscellaneous food additives 41

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 16-24 June 1971

Members invited by FAO :

Dr T. Cayle, Director of Research, Wallerstein Company, New York, N.Y., USA
Mr H. Cheftel, Chairman, Scientific Sub-Committee, Permanent International Committee on Canned Foods, Paris, France
Dr H. Egan, Government Chemist, Department of Trade and Industry, London, England (*Vice-Chairman*)
Dr K. Kojima, Chief, Food Chemistry Division, Ministry of Health and Welfare, Tokyo, Japan
Mr H. P. Mollenhauer, Chief, International Food Section, Federal Ministry of Youth, Family and Health, Bad Godesberg, Federal Republic of Germany (*Rapporteur*)
Dr L. Schinetti, Director, Research Department, Società del Plasmon S.p.A, Milan, Italy

Members invited by WHO : *

Professor Bernard Blanc, Director, Swiss Federal Dairy Science Institute, Liebefeld, Bern, Switzerland
Dr H. Blumenthal, Deputy Director, Division of Toxicology, Bureau of Foods, Food and Drug Administration, Washington, D.C., USA (*Rapporteur*)
Dr A. P. de Groot, Head, Department of Biology and Toxicology, Central Institute for Nutrition and Food Research, TNO, Zeist, Netherlands
Professor M. J. Rand, Department of Pharmacology, University of Melbourne, Victoria, Australia (*Chairman*)
Professor R. Truhaut, Director, Toxicological Research Centre, Faculty of Pharmacy, University of Paris, France

Observers (invited by FAO) :

Miss O. Demine, European Community Commission, Brussels, Belgium
Mr D. F. Dodgen, Director, Food Chemicals Codex, National Academy of Sciences, Washington, D.C., USA
Professor M. J. L. Dols, International Union of Nutritional Sciences, Wassenaar, Netherlands
Dr H. Lange, Chairman, Food Chemistry Division, Society of German Chemistry, Frankfurt am Main, Federal Republic of Germany
Dr R. Morf, Secretary-General, International Union of Pure and Applied Chemistry, Zurich, Switzerland
Mr G. F. Wilmink, Chairman, Codex Committee on Food Additives, The Hague, Netherlands

Secretariat :

Dr C. Agthe, Chemical Carcinogenesis Unit, International Agency for Research on Cancer, Lyon, France
Dr P. E. Berteau, Scientist, Food Additives, WHO, Geneva, Switzerland
Dr P. S. Elias, Principal Medical Officer, Toxicology, Department of Health and Social Security, London, England (*Consultant*)
Professor F. V. Kosikowski, Professor of Food Science, Department of Food Science, Cornell University, New York, USA (*Consultant*)
Dr L. G. Ladomery, Food Standards Officer, FAO/WHO Food Standards Programme, FAO, Rome, Italy
Dr F. C. Lu, Chief, Food Additives, WHO, Geneva, Switzerland (*Joint Secretary*)
Mr R. K. Malik, Food Policy and Food Science Service, Nutrition Division, FAO, Rome, Italy (*Joint Secretary*)
Dr F. D. Tollenaar, Foreign Projects Service, Organization for Nutrition and Food Research, TNO, The Hague, Netherlands (*Consultant*)

---

* Unable to attend : Dr A. N. Zajcev, Head of the Laboratory for Research on the Health Aspects of Food Additives, Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow, USSR.

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 of the Joint FAO/WHO Expert Committee on Food Additives

A Joint FAO/WHO Expert Committee on Food Additives met in Rome from 16 to 24 June 1971. The Meeting was opened by Mr E. M. Ojala, Assistant Director-General, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization.

1. INTRODUCTION

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955,[1] fourteen Joint FAO/WHO Expert Committees on Food Additives have met (see Annex 1). An explanatory note on the activities of the Joint FAO/WHO Expert Committee on Food Additives is given in Annex 4 to the tenth report of the Committee.[2] This annex sets out the general principles that govern the establishment of specifications for food additives and explains the detailed construction of the specifications themselves. It also describes the biological data on which the reports are based together with the factors that are taken into account in the toxicological evaluations.

The present meeting was convened on the recommendations made in the fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Its terms of reference were: (1) to assess the use of additives in baby foods, (2) to make toxicological evaluations of certain food additives and to draw up specifications for them, and (3) to review the technolog-

---

[1] *FAO Nutrition Meetings Report Series*, 1956, No. 11; *Wld Hlth Org. techn. Rep. Ser.*, 1956, No. 107.

[2] See Annex 1, ref. 13.

ical efficacy of certain antioxidants (see Annex 2). Most of the substances considered had been suggested by the Codex Committee on Food Additives, to which the Expert Committee acts as an advisory body on questions of toxicity, specifications for identity and purity, and methods of analysis.

In order to facilitate the discussions, the Committee constituted itself into two groups, one of which gave major attention to toxicological evaluations and the other to chemical specifications and technological efficacy.

2. THE PROBLEM OF ADDITIVES IN BABY FOODS

The Committee considered a draft report prepared by an FAO/WHO Meeting on Additives in Baby Foods, which had been held from 14 to 16 June 1971. This Meeting had been convened with the following terms of reference :

(*a*) to set forth the principles to be considered in accepting the use of additives in baby foods ;

(*b*) to draw attention to the need for specific toxicological approaches to the evaluation of the safety of food additives in infant formulas and baby food ;

(*c*) to discuss a number of additives in respect to which specific queries had been raised.

The Meeting made a distinction between baby foods suitable for infants up to the age of 12 weeks and those designed to be given to the older infant. This distinction was based on the argument that for a variety of physiological reasons the very young infant is relatively more vulnerable. The Meeting also set forth certain more stringent testing procedures that, it was suggested, should be employed when considering the acceptability of food additives in baby foods. Finally, the Meeting considered briefly the general principles to be borne in mind when considering the technological aspects of food additives in baby foods.

The Committee discussed in detail the various points raised in the draft report and agreed on certain modifications. It was accepted that this report reflected the increasing interest in infant and baby foods and that much basic research was necessary, particularly on the correlation of human development in infancy with that of test animals, before adequate evaluation along the lines suggested by the Group could be made. The Committee also stressed that the presence of residues of environmental contaminants in all sources of infant nutrition raised a number of problems that would require further exploration. The Committee adopted the report of the Meeting as modified in the light of the discussions and recommended that the modified version be annexed to the present report (see Annex 3).

3. PRINCIPLES GOVERNING CHEMICAL SPECIFICATIONS

3.1 Scope

The general principles governing the elaboration of specifications have been summarized in Annex 4 of the tenth report.[1] The specifications have been developed for the use of toxicologists and others concerned with the identity and purity of the additives and to prescribe an adequate degree of purity that should be met. References are made in the individual specifications to some of the criteria that may be of interest in commerce. However they do not only include requirements of interest to a commercial user. The specifications are meant to be of use at an international level; to the extent that the necessary data were available they have therefore been drawn up in such a way as to cover suitable products manufactured in various parts of the world.

As in previous meetings, the Committee agreed that specifications would be drawn up only for substances that are manufactured commercially and that have been recognized by the Committee as being of use in the food industry.

3.2 Composite products

Specifications are normally prepared for the pure substance that can be described in chemical terms. There are, however, composite products, such as enzyme preparations, that contain one or more active components as well as diluents, preservatives, antioxidants, and other substances used in food processing. In such cases, the variety of formulations used may not allow the drawing up of complete specifications for each individual product. In order to assure toxicological safety and the standard of purity required for such products in accordance with good manufacturing practice, general specifications may be prepared, setting out a number of criteria that would encompass all closely related products of the type in question. Wherever possible, additional requirements for individual products may be laid down in a specification; these are to be read in conjunction with the general specification. This procedure has been followed for enzyme preparations (see section 5.1.1).

3.3 Identity of the substance

In some cases it is difficult to establish the precise identity of the substance that has been used in toxicological experiments. Doubt may exist as to the extent to which the specifications elaborated on the basis of an

[1] See Annex 1, ref. 13.

equivalent product available in commerce would meet the toxicological requirements that have been established. This is further evidence of the need for adequate information on the identity of the material used.

There appears to be some difficulty in determining the level of use of certain preparations, especially those of the types mentioned in sections 3.2 and 5.3.1, because they may be manufactured at varying concentrations. In such cases more data are required on the actual composition and formulation and on the levels of use in specific foods.

3.4 Development and improvement of analytical methods

Analytical methods are continually being developed to improve sensitivity, specificity, accuracy, and reproducibility. The need for new and improved methods is particularly great with composite substances, such as enzyme preparations. The methods cited in the specifications were, however, considered to be applicable to the analysis of products moving in international commerce.

For enzyme preparations the content of the pure enzyme is not specified, only the enzymatic activity; therefore the analysis is limited to the assay of potency on selected substrates, expressed as activity per unit weight of the preparation.

4. PRINCIPLES GOVERNING TOXICOLOGICAL EVALUATIONS

The Committee again adopted the same general principles for the establishment of acceptable daily intakes (ADIs) set out in previous relevant reports of the Joint FAO/WHO Expert Committee on Food Additives. The definitions of ADIs have already been stated in the thirteenth report of the Committee.[1] Emphasis was placed on the more recent advances in toxicological and biochemical methodology and interpretation set forth in the report of the WHO Scientific Group on Procedures for investigating Intentional and Unintentional Food Additives.[2]

Many of the substances on the agenda of this meeting were not considered to require definite ADIs, their use being limited by " good manufacturing practice ". In this connexion the Committee took the view that good manufacturing practice in the use of additives must be interpreted as the use of additives in a manner that fully respects the considerations set out in the first report. These include the maintenance of nutritional quality, reduction of wastage by the improvement of keeping quality, attractiveness to the consumer, and avoidance of misuse, e.g., for the purpose of disguising

[1] See Annex 1, ref. 19.

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

faulty processing or handling techniques or otherwise deceiving the consumer.

The Committee recognized that the subject of good manufacturing practice was of great importance and recommends that it be given more detailed consideration in the future.

A number of special considerations relating to enzymes and modified starches are given in section 5.

5. COMMENTS ON SUBSTANCES ON THE AGENDA

Most of the substances on the agenda (see Annex 2) are considered in detail in the individual specifications and monographs. These substances were considered under three main headings : enzymes, modified starches, and a group of miscellaneous compounds. The following sections summarize the results of the deliberations of the Committee on all the substances on the agenda.

5.1 Enzymes

5.1.1 *General remarks*

(*a*) *Technological considerations*

Enzymes are used in food processing because they act under mild conditions, in a specific manner, and selectively on certain components of the food. The enzymes may remain intact or they may be inactivated, denatured, or removed when the desired change has been achieved.

Enzyme preparations have been grouped according to their source. Within these groups they have been further subdivided according to their general substrate specificity. The recommendations of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes, according to substrate specificity alone, relate only to pure enzymes and are not applicable to commercial enzyme preparations used in food technology.

These enzyme preparations are generally not single enzymes but often have enzymatic activities besides that of the main principle and also contain non-enzymatic proteins, metabolites, and other residues from the source material. In addition, residues of substances used in their manufacture and adjuncts such as buffers, inorganic salts, diluents, or stabilizers are often present. Consequently, for toxicological evaluation the expression of the amount of the enzyme preparation used in terms of activity is less relevant than a statement in terms of weight. For technological reasons, such as efficiency and ease of application, enzyme preparations are used over a

wide range of activities and dilutions. Commercial enzyme preparations are usually used in concentrations below 5% and in many cases far below 0.1%.

A general specification pertaining to all enzyme preparations used in food processing has been prepared. This is supplemented by specifications for individual enzyme preparations that cover the characteristics not included in the general specification.

Analytical methods are recommended for determining the activity of each enzyme preparation. Some of the methods call for the use of standard reference materials. The Committee strongly recommends that such reference standards be made available on an international basis. Methods are available for estimating residual antibiotic activity, but information is needed on the extent to which these are applicable to enzyme preparations. A suitable method is available for estimating the aflatoxin content. Should other toxins of concern be discovered, it will be necessary to develop control methods for them.

In the specifications the individual species of micro-organisms from which enzyme preparations are derived are named, but the specifications contain no means of identifying the source organism in the finished enzyme preparation. The Committee recommended that methods be developed to remedy this deficiency.

(*b*) *Toxicological considerations*

The increasing use of enzymes in food technology has made it imperative that consideration be given to the toxicological evaluation of enzymes as a class of food additives. Only those enzyme preparations that are known to have a definite use in food technology were considered. Enzymes with purely therapeutic, animal feed, or industrial use, unrelated to food technology, were outside the terms of reference of the Committee, but in making toxicological evaluations pertinent observations based on the human therapeutic use of certain enzyme preparations were taken into account.

The Committee took a cautious view of the possible hazard arising from the presence of toxins associated with enzyme preparations from micro-organisms, particularly with reference to mycotoxins. In recognition of the considerable differences in metabolic behaviour exhibited by individual species within a genus, the Committee considered it inadvisable to accept specification by genus alone.

The enzymes and other proteins present in enzyme preparations may give rise to allergic reactions following inhalation or dermal application. When they reach the consumer in the form of food additives, the enzymes would only be ingested. Consequently allergic reactions would seem unlikely to occur to any greater extent than they do with other protein constituents of food. Certain enzymes are used in human medicine. Large oral doses

of these enzymes or their parenteral administration has a distinct tendency to produce allergic hypersensitivity. Patients treated with enzymes are especially at risk to allergic reactions from the use as food additives of enzyme preparations containing the same enzymes as those used in therapeutics. Because of this risk the Committee took the view that the use in food manufacture of enzymes antigenically related to those used in therapeutics should be avoided.

The Committee emphasized the need to adhere rigorously to the principles of good hygienic and manufacturing practices, and to the general specifications for enzyme preparations for food processing elaborated during the meeting. During the discussion of individual enzyme preparations the Committee developed some guidelines for arriving at evaluations. The Committee considered that the source of the enzyme preparations provides a useful indication of whether a potential toxicological hazard exists.

5.1.2 *Individual enzymes*

A number of sources of enzymes were recognized.

(*a*) Enzymes obtained from edible tissues of animals commonly used as foods were regarded as foods. Consequently they were acceptable and no monographs on these enzymes were prepared. Specifications were drawn up for the following animal enzyme preparations : rennet,[1] bovine rennet,[2] pepsin, trypsin, animal lipase, and catalase (bovine liver).

(*b*) Enzymes obtained from the normally edible portions of plants were likewise regarded as foods. Those considered to fall within this category were malt, bromelain, and papain. The Committee was informed that the commercial source of papain for food additive use is the unripe fruit of *Carica papaya* L. However, since ripe fruit also contains papain the unripe fruit can be considered as equivalent to an edible portion of the plant ; in some parts of the world the cooked unripe fruit is eaten.

The available information indicated that commercial enzyme preparations of ficin are obtained from non-edible parts of the plant. As no toxicological data were available, a decision on its acceptability was postponed and no monograph was prepared. However, a tentative specification for ficin was prepared.

(*c*) Enzymes derived from micro-organisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods were themselves regarded as foods. Enzymes in this category were those derived from *Saccharomyces* species, *Lactobacillus* species, and lactic

[1] Prepared from the fourth stomach of calves, lambs, or kids.

[2] Prepared from the fourth stomach of bovines, sheep, or goats.

acid *Streptococcus* species. A specification was drawn up for carbohydrase prepared from *Saccharomyces* species. *Rhizopus oryzae* varieties are also in this category and a specification was prepared for microbial carbohydrase prepared from *Rhizopus oryzae* varieties.

The Committee was aware that *Aspergillus oryzae* varieties are used in certain parts of the world in the preparation of food. However, one of the known metabolites of *Aspergillus oryzae* is β-nitropropionic acid.[1] According to some preliminary experimental findings, this substance may have carcinogenic activity in mice.[2] Consequently evaluation of the enzyme preparations from this organism was postponed, although tentative specifications were prepared for a carbohydrase, a protease, and a lipase.

Since a metabolite of *Aspergillus oryzae* is suspected of carcinogenic potential, there is an urgent need to investigate the matter. Information is required on the culture conditions under which this metabolite is formed, the amounts formed under commercial conditions of culturing, and the amounts present in the enzyme preparations of commerce. A suitable method of assay for β-nitropropionic acid is required and further toxicological investigations should be instituted. This problem with *Aspergillus oryzae* calls attention to the need for broader studies on the nature of the metabolites produced by other micro-organisms used for the production of enzyme preparations.

(*d*) Enzymes derived from non-pathogenic micro-organisms commonly found as contaminants of foods were not considered in the same light as those regarded as food.

The micro-organisms falling into this group were *Bacillus subtilis* varieties, *Bacillus cereus*, and *Aspergillus niger* varieties. The Committee accepted, on the basis of the available data, that the food additive use of enzyme preparations from *Bacillus subtilis* varieties required no limitation except for good manufacturing practice. A monograph and specification were prepared for the mixed microbial carbohydrases and proteases. It was recognized that commercial enzyme preparations invariably contained both protease and carbohydrase activity. Since the relevant toxicological research had been conducted on the substances used in commercial practice, it was agreed that enzyme preparations having both activities could be used. Data on enzyme preparations that are currently in use could be taken to apply to more purified preparations of individual enzymes. The evaluation of enzyme preparations from *Bacillus cereus* was postponed because

---

[1] Miller, M. W., ed. (1961) *The Pfizer Handbook of Microbial Metabolites*, New York, McGraw Hill.

[2] Kinosita, R., Ishiko, T., Sugiyama, S., Seto, T., Igarasi, S. & Goets, I. E. (1968) *Cancer Res.*, **28**, 2296.

of the close relationship of this organism to the *Anthrax bacillus*.[1] A tentative specification was drawn up for microbial rennet prepared from *Bacillus cereus*. The Committee agreed that, on a temporary basis, no limit was required for microbial carbohydrase and microbial glucose oxidase preparations from *Aspergillus niger* varieties, except for good manufacturing practice, but that this temporary acceptance should be subject to the results of further short-term tests. Tentative specifications were drawn up for these two enzymes and for catalase prepared from *Aspergillus niger* varieties.

(*e*) Enzymes derived from less well known micro-organisms were considered by the Committee to require more extensive toxicological studies. Monographs were drawn up for microbial rennets prepared from *Mucor miehei, Mucor pusillus,* and *Endothia parasitica,* and for microbial catalase prepared from *Micrococcus lysodeikticus*. For all these preparations two-year studies in rats are required, but the Committee agreed that, on a temporary basis, no limit was required for the rennet preparations from these three species, except for good manufacturing practice. In the case of *Micrococcus lysodeikticus* no evaluation was made. Tentative specifications were prepared for microbial rennet (*Mucor* sp. — *M. miehei* and *M. pusillus*) ; microbial rennet (*Endothia parasitica*) ; and catalase (*Micrococcus lysodeikticus*).

No monographs were drawn up for microbial rennet prepared from *Irpax lacteus,* for microbial glucose oxidase prepared from *Penicillium amagasakiense,* or for a carbohydrase prepared from *Arthrobacter,* as no specifications were available. A number of other enzyme preparations were not considered because they were identified by trade name only and no information as to the source of the toxicological properties was available.

5.2 Modified starches

At the thirteenth meeting of the Committee [2] specifications were prepared for some modified starches. The present meeting reviewed these specifications in the light of comments received and made some minor corrections. Amended specifications will be published, superseding the earlier ones.

Very little information was available on starch sodium succinate, starch sodium octenyl succinate, starch aluminium octenyl succinate, sodium carboxymethyl distarch glycerol, and acetylated distarch adipate ; these products are assumed to be of no commercial significance for food additive use and were not considered further.

---

[1] *Bergey's Manual of Determinative Bacteriology* (1967, 7th ed., Baltimore, Williams & Wilkins, p. 618) states : " Smith, Gordon and Clark (U.S. Dept. Agr. Misc. Pub. 559, 1946, 54) considered *Bacillus anthracis* as a pathogenic variety of *Bacillus cereus* because certain strains of the two were in close agreement in all characters except pathogenicity. As strains of *Bacillus anthracis* may become avirulent, and as certain strains of *Bacillus cereus* may be lethal in massive dosages (Clark, Jour. Bact., 33, 1937, 435) the relationship is closer than most investigators realize."

[2] See Annex 1, ref. 19.

5.2.1 *General considerations*

In its consideration of the modified starches the Committee took into account the various reservations expressed in the thirteenth report. However, the Committee had before it a large amount of new data which included long-term and reproduction studies on a number of modified starches. Since these modified starches are closely related, it was felt that data from the starches subjected to multiple treatment could be applied to the starches given single treatment and *vice versa.*

The Committee also considered the need for providing data on metabolic studies in man. It was felt that sufficient information was now available from extensive animal studies to permit evaluations to be made without data on human metabolism. Nevertheless more studies in man would be desirable to corroborate the extrapolations based on animal data.

The Committee noted that, with the exception of modification by phosphate, all the modifications of starches under review appeared to result in some resistance to complete hydrolysis in the gastrointestinal tract and that this may be associated with the presence of novel products of digestion. It seems probable that novel products of digestion from the modified starches evaluated would be produced only in small amounts, since the degree of modification of these starches is slight and they are well utilized when fed in moderate amounts. The question of whether certain sections of the human population show special susceptibility to novel products of digestion is partly answered by the absence of deleterious effects in the long-term and reproduction studies. No doubt starches with greater degrees of modification might give rise to problems associated with novel products of digestion, as indicated in the thirteenth report.[1]

The Committee noted that, with the exception of phosphated starches, in the group of modified starches considered dietary levels exceeding 10% tended to produce diarrhoea and caecal enlargement unassociated with any histopathological abnormalities in test animals. These findings may be of little toxicological significance, however, since unmodified starches also cause diarrhoea and caecal enlargement. When starches comprise a high proportion of the diet, as in the animal feeding tests, they provide a large bulk of intestinal content by virtue of their hydrophilic nature and the amount present may exceed the capacity of digestive enzymes to produce complete hydrolysis, especially as the transit time through the small intestine would be decreased due to the stimulation of peristaltic activity by increased filling. Loose stools and diarrhoea are normal physiological consequences of increased bulk of contents in the colon and rectum, and caecal enlargement could be a functional hypertrophy arising as a normal consequence of increased bulk of caecal contents. Furthermore, feeding

[1] See Annex 1, ref. 19.

tests with large amounts of starches would produce changes in the composition of the intestinal contents and a consequent change in intestinal flora, which too could lead to diarrhoea. It might be desirable, however, to study these phenomena more fully. Since modified starches do not exist technologically in a form other than that having been processed by cooking, the possibility of persorption is irrelevant in relation to the evaluation of modified starches used in food.

In view of the above considerations, the Committee believed that it was no longer necessary to establish definite acceptable daily intakes for the group of modified starches under consideration and agreed to the use of these compounds being limited only by good manufacturing practice. However, such acceptance of the modified starches would be on a temporary basis, until the further information required is available.

5.2.2 *Individual starches*

The evaluations arrived at are summarized in Annex 5 and discussed in the individual monographs. Of the individual modified starches on the agenda, the distarch phosphates prepared by two separate methods were combined into a single monograph. As evaluation of these modified starches was based partly on information on phosphated distarch phosphate, the Committee also reconsidered this modified starch, although it was not on the agenda.

Hydroxypropyl distarch phosphate could not be evaluated owing to the lack of adequate data, but a monograph was prepared to indicate specific needs for further testing.

For oxidized starch, starch acetate, hydroxypropyl starch, hydroxypropyl distarch glycerol, distarch phosphate, phosphated distarch phosphate, acetylated distarch phosphate, distarch glycerol, acetylated distarch glycerol, and acetylated distarch adipate, the available toxicological data were adequate for assigning temporary approval for use, limited by good manufacturing practices.

5.3 Miscellaneous food additives

5.3.1 *Caramel colours*

The subject of caramel colours was considered by the thirteenth meeting of the Joint FAO/WHO Expert Committee on Food Additives, which concluded that, with the exception of those caramels prepared by using ammonia or ammonium salts, caramel and caramel colours are natural constituents of the diet and are acceptable as additives. The present Committee endorsed this opinion with reference to caramel colours not made by the ammonia process and in addition gave more detailed consideration to those made by the ammonia process, in which traces of

4-methylimidazole and other nitrogen-containing heterocyclic compounds are formed.

The formation of these heterocyclic compounds in glucose-ammonia reactions is a function of the ratio of the reactants : the higher the molar ratio of ammonia to glucose, the greater will be the proportion of heterocyclic compounds present in the final caramel product. Other reaction conditions can also influence the proportion of heterocyclic compounds formed.

Different grades of caramels are sometimes described as being " single strength " or " double strength ", based on their tinctorial capacity. The amounts of fixed nitrogen or sulfur dioxide are higher in " double strength " caramels. It is understood that " double strength " caramels are used in amounts equal to or less than one-half the amounts of " single strength" caramels to achieve the same colour effect in the final food product.

A tentative specification has been prepared for caramels that are produced with ammonium compounds as processing adjuncts, because of the toxicological significance of the nitrogen-containing heterocyclic compounds that are formed and because of insufficient information on the amounts of these compounds present in relation to the strength of colour. A revised specification was prepared for other types of caramels produced without using ammonium compounds.

Pending more precise information about the terms " single strength " and " double strength " and their tinctorial capacity, the Committee considered it advisable to prepare the specification and to evaluate caramels in terms of the European Brewing Convention (EBC) colour units.[1]

A number of short-term feeding studies with caramel colours made by the ammonia process were available as well as analytical data on the levels of 4-methylimidazole. Although these studies indicated little or no toxicity the Committee felt there was a need to investigate the long-term effects of 4-methylimidazole and related nitrogen-containing heterocyclic compounds. Since many of these minor contaminants have not yet been fully identified, it was also felt that the proposed further studies should be done on a caramel colour made by the ammonia process rather than on 4-methylimidazole *per se*. On the basis of the data available, the Committee was able to establish a temporary acceptable daily intake for caramel colours produced by the ammonia process, as given in the relevant monograph.

5.3.2 *Choline salts*

These were included on the agenda because of their apparent use as salt substitutes. However, the Committee had no information on this particular

[1] See : *Appendix B — Method for measurement of colour intensity*. In : *Specification for caramel for use in foodstuffs, B.S. 3874 : 1965*, London, British Standards Institution. This method is based on Method 304E of the European Brewery Convention.

use nor any indication of the particular choline salts that were being used. No specifications were therefore prepared. The Committee was informed that some choline salts are constituents of salt-substitute mixtures; some others are added to certain alcoholic beverages because of their effect on flavour. There may be a place for the use of choline salts in low-sodium diets.

Choline is present in animals as part of many physiologically important compounds. It has well recognized pharmacological effects when given by injection, but is without appreciable adverse action when given orally. Considerable experience has been gained from the use of choline and its various salts in the treatment of human diseases. Furthermore, choline, in free and combined form, is a natural constituent of many foods.

The limited additional intake, when choline is used as a food additive, presents no toxicological hazards, and as the intended uses of choline salts were not well defined the Committee deemed it unnecessary to prepare a monograph on these compounds. In the opinion of the Committee, the acids used to form choline salts should be only those approved for food additive use.

5.3.3 *Dimethylpolysiloxane*

This substance was on the agenda for review of further toxicological evidence, having been considered already by the thirteenth Joint FAO/WHO Expert Committee on Food Additives which prepared a specification. However, as no relevant new information was forthcoming the Committee decided that the previous evaluation should stand and no monograph has been prepared. The Committee was informed that the metabolic studies requested at the thirteenth meeting would be available in the near future.

5.3.4 *Hexamethylenetetramine*

Specifications for this substance prepared by the tenth Joint FAO/WHO Expert Committee on Food Additives were reviewed. No changes were made and the specifications will be published.

The technological efficacy of a number of other antimicrobial agents was evaluated at the fourteenth meeting of the Joint FAO/WHO Expert Committee on Food Additives. At that time no assessment could be made for hexamethylenetetramine because relevant toxicological investigations had not been completed. Many new toxicological data have since become available. When considering these data, the Committee placed most weight on the findings after oral administration, which allowed the assignment of a temporary acceptable daily intake, pending the completion of a teratological study in dogs.

An opportunity was also taken to discuss the technological efficacy of hexamethylenetetramine. Means of processing certain foods without the

use of hexamethylenetetramine have been developed in recent years, and this may limit its application in these foods.

5.3.5 *Esters of glycerol and thermally oxidized soya bean fatty acids; thermally oxidized soya bean oil*

The Committee had information on two distinct products used for two different purposes; one was an emulsifier, the other a release agent.

A tentative specification for esters of glycerol and thermally oxidized soya bean fatty acids, which are used as emulsifiers, was prepared and will be published. Additional information is required on description, identification, and purity tests. The need for unambiguous specifications was stressed, since it has been shown that the toxicity is a function of the degree of aeration and the duration of heating employed in their manufacture.[1] On the basis of the toxicological information available, the Committee established a temporary ADI for these esters.

The evidence on thermally oxidized soya bean oil was inadequate to permit the establishment of an ADI. A tentative specification was prepared but will be available only on request.

5.3.6 *Microcrystalline cellulose*

A specification was prepared by the Committee for microcrystalline cellulose and will be published. In evaluating the available information, the close relationship of microcrystalline cellulose to naturally occurring cellulose, a natural constituent of foods, was taken into account. Because the physical form of microcrystalline cellulose changes on contact with water the Committee considered that no problem of persorption arises. In addition, the Committee took note of the prior evaluations of several modified celluloses.[2] It was concluded that no limitation other than that imposed by good manufacturing practice was needed for the use of microcrystalline cellulose as an additive.

5.3.7 *Propylene glycol alginate*

A specification for this substance was prepared at the thirteenth meeting of the Committee.[3] However, there is a lack of information on the extent of the *in vivo* hydrolysis of propylene glycol alginate by digestive enzymes. There is evidence that some hydrolysis occurs with liberation of propylene glycol, but whether there is any intake of propylene glycol from this source could not be determined with certainty. When computing the intake of propylene glycol from all other sources, allowance must be made for the

---

[1] Artman, N. R. (1969) *Advanc. Lip. Res.*, 7, 245-330.

[2] See Annex 1, ref. 13 and 19.

[3] See Annex 1, ref. 21.

amount of propylene glycol that could be liberated from the alginate ether. With this restriction an unconditional acceptable daily intake was established.

5.3.8 *Stearoyl lactylic acid, calcium and sodium salts*

These substances have been reviewed previously and tentative specifications were prepared at the thirteenth meeting of the Committee.[1] These have now been finalized and will be published. Previously there had been concern over the significance of some toxicological observations. However, stearoyl lactylates act in the same way as equivalent amounts of stearic acid and lactic acid when these are administered concomitantly. The production of " lipogranulomata " in animals fed high levels of stearoyl lactylates can be prevented by incorporation of additional unsaturated fatty acids into diets. This effect therefore appears to be the result of dietary imbalance and there may be a need to take into account the intake of stearic acid from all sources. The acceptable daily intake established was higher than that assigned in the previous evaluation.

5.3.9 *Tin and stannous chloride*

A specification for stannous chloride was prepared at the fourteenth meeting of the Committee.[2] Information is lacking on the various combinations in which tin is present in canned foods and beverages. The uptake of tin from the container by canned foods is a complex reaction, which may be influenced by the presence of ions such as nitrate, oxalate or potassium in the product. Further research should be directed toward determining the form in which tin is present in beverages, such as diluted citrus juices, and in canned foods, such as fruit and vegetables, where it is apparently bound to polyphenolic compounds or to proteins. Priority should be given to consideration of tin in beverages since it is these that have been reported to cause acute gastrointestinal troubles. The study of tin in canned foods is relevant to possible long-term toxic effects. The effect of tin on the organoleptic, keeping, and other qualities of foods is not fully understood and should be examined more thoroughly.

After extensive discussion of the significance of new laboratory data and further human epidemiological observations, the previous evaluation was confirmed, i.e., no ADI could be assigned, but there was no need to depart from the limits set by good manufacturing practice. However, further assessment will be needed when the results of work in progress become available.

---

[1] See Annex 1, ref. 19.

[2] See Annex 1, ref. 24.

6. REVIEW OF THE TECHNOLOGICAL EFFICACY OF SOME ANTIOXIDANTS

At previous meetings the Committee prepared specifications for, and made toxicological evaluations of, a number of antioxidants and synergists. At this meeting, the Committee carried out a review of the technological efficacy of these substances and prepared monographs on the following: ascorbic acid, sodium ascorbate, ascorbyl palmitate, isoascorbic acid, sodium isoascorbate, butylated hydroxyanisole, butylated hydroxytoluene, propyl gallates, octyl gallates, dodecyl gallates, thiodipropionic acid, dilauryl thiodipropionic acid, tocopherols, calcium disodium ethylenediaminetetraacetate, disodium ethylenediaminetetraacetate, citric acid, isopropyl and stearyl citrate, tartaric acid, and phosphoric acid.

The monographs do not contain recommendations for use, legal strictures, or clearances, but constitute a review of scientific data available in the literature. The use levels given in the monographs do not necessarily correspond to those specified by legislation or to the optimum concentrations for technological purposes.

The review of antioxidants comprises general considerations followed by individual monographs on 13 substances. No monograph was prepared on ascorbyl stearate because of inadequate information.

6.1 Methods of analysis of antioxidants in food

The Committee decided to follow the earlier practice and not elaborate methods of analyses for determining the additives in food as this is being done by the Joint FAO/WHO Codex Alimentarius Commission.

6.2 Review of efficacy

Antioxidants are used in food processing to prevent a variety of undesirable changes caused by oxidation, including the following: rancidity in fats, oils, and fat-containing foods; discoloration in meat and meat products; enzymatic browning in fruit and vegetables; oxidation defects in citrus juices. The organoleptic deterioration may be accompanied by loss of nutritive value and the generation of toxic materials.

The review deals mainly with antioxidants used for preventing fat oxidation. These do not provide protection against oxidation in all cases. More precise knowledge about the mechanisms of oxidation and of antioxidant activity is required. More information is also required about the efficacy of antioxidants and methods of testing for efficacy, as accelerated tests, which are frequently used, give results that do not always correlate with the outcome of commercial storage tests.

It was considered that the use of antioxidants should be related to the possibility of preventing oxidative changes in foods by other means, such

as gas packing or avoidance of copper contamination in accordance with good manufacturing practice.

7. RECOMMENDATIONS

1. FAO and WHO should convene further meetings of the Joint FAO/WHO Expert Committee on Food Additives to deal with additives that have not been previously considered by the Committee and those additives that have been considered but on which new data have become available with respect to (*a*) evaluation of potential health hazards, (*b*) preparation of specifications, and (*c*) reviews of technological efficacy.

2. WHO should promote fundamental research in the areas set out in Annex 3, including toxicological investigations on food additives that are required in baby foods.

3. FAO and WHO should consider in greater detail the concept of good manufacturing practice with a view to developing working definitions or criteria. This is important for both toxicological and technological considerations.

4. FAO should encourage studies on the various complexes in which tin occurs in canned foods and beverages, as well as on the effects of tin on the organoleptic, keeping, and other qualities of foods.

5. FAO and WHO should encourage research into methods for identifying source organisms from which commercial enzyme preparations are obtained.

6. FAO and WHO should encourage studies on the nature and the toxicity of the metabolites occurring in culture media from which commercial enzyme preparations are obtained.

7. FAO should designate an international centre from which reference standards of enzyme preparations could be more generally available.

8. When further toxicological information is required by the Committee it is in the interest of public health to obtain this information expeditiously. Accordingly it is recommended that WHO should make these requirements known as soon as possible to those with the necessary expertise in the appropriate area of research.

9. It is recommended that appropriate action be taken by FAO and WHO to reissue promptly reports, specifications, and monographs that are out of print.

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*, 1956, No. 11: *Wld Hlth Org. techn. Rep. Ser.*, 1956, No. 129.
2. Procedures for the Testing of International 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 Colors*, 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 some Food Colours, *FAO Nutrition Meetings Report Series*, 1966, No. 38B; WHO/Food Add/66.25.

---

* 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.

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.

* 12. Toxicological Evaluation of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-treatment Agents, Acids and Bases, *FAO Nutrition Meetings Report Series*, No. 40 A, B, C ; WHO/Food Add/ 67.29.

13. 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.

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 and Stabilizers, Anticaking Agents and Certain Other Substances. *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. 48 A ; WHO/Food Add/ 71.39.

---

* 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.

* 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/71.40.

* 25. A Review of the Technological Efficacy of Some Antimicrobial Agents. *FAO Nutrition Meetings Report Series*, 1971, No. 48C ; WHO/Food Add/ 71.41.

* 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.

Annex 2

LIST OF SUBSTANCES ON THE AGENDA

1. *Enzymes used in food technology*

2. *Modified starches used as food additives*

   Oxidized starches
   Starch acetate
   Hydroxypropyl starch
   Distarch phosphate (phosphorus oxychloride)
   Acetylated distarch phosphate
   Hydroxypropyl distarch phosphate
   Distarch glycerol
   Acetylated distarch glycerol
   Hydroxypropyl distarch glycerol
   Acetylated distarch adipate
   Starch sodium succinate
   Starch sodium octenyl succinate
   Starch aluminium octenyl succinate
   Sodium carboxymethyl distarch glycerol

3. *Miscellaneous substances*

   Caramel
   Choline salts (used as salt substitutes)
   Hexamethylenetetramine
   Esters of glycerol and thermally oxidized soya bean fatty acids
   Microcrystalline cellulose
   Propylene glycol alginate
   Stearoyl lactylate
   Tin

4. *Antioxidants and antioxidant synergists* (for review of technological efficacy only)

   Ascorbic acid, isoascorbic acid, and their sodium salts
   Ascorbyl palmitate and ascorbyl stearate
   Butylated hydroxyanisole (BHA)

Butylated hydroxytoluene (BHT)
Gallates—dodecyl, octyl, and propyl
Thiodipropionic acid and dilauryl thiodipropionate
Tocopherols—alpha and mixed concentrate
Synergists :

Citric, tartaric, and phosphoric acids
Ethylenediaminetetraacetate, calcium disodium
Ethylenediaminetetraacetate, disodium
Stearyl citrate
Isopropyl citrate

Annex 3

ADDITIVES IN BABY FOODS

Report of an FAO/WHO Meeting

CONTENTS

	Page
1. Introduction	29
2. General considerations	29
2.1 Scope	29
2.2 Principles for the use of intentional food additives	30
2.3 Pesticides and contaminants	31
2.4 Other general considerations	31
3. Technological considerations	31
3.1 Chemical preservatives	32
3.2 Food colours	32
3.3 Emulsifiers, stabilizers, and thickeners	32
3.4 Flavouring agents and flavouring enhancers	32
3.5 Antioxidants	32
3.6 Buffers and pH regulators	33
4. Toxicological considerations	33
4.1 Vulnerability of very young infants	33
4.2 Toxicological testing	34
5. Specific food additives	35
5.1 Modified starches	35
5.2 Lactic acid	35
5.3 Nitrates and nitrites	35
5.4 Phosphates and calcium	36
5.5 Sodium compounds	36
6. Recommendations	37

FAO/WHO MEETING ON ADDITIVES IN BABY FOODS

Rome, 14-16 June 1971

Members :

Dr W. T. C. Berry, Principal Medical Officer, Nutrition, Department of Health and Social Security, London, England

Dr P. S. Elias, Principal Medical Officer, Toxicology, Department of Health and Social Security, London, England (*Chairman*)

Mr G. Loggers, Public Health Inspector, Ministry of Social Affairs and Public Health, Leidschendam, Netherlands

Mr H. P. Mollenhauer, Chief, International Food Affairs Section, Federal Ministry of Youth, Family and Health, Bad Godesberg, Federal Republic of Germany (*Vice-Chairman*)

Dr K. Murray, Chief, Nutrition Division, Food and Drug Directorate, Ottawa, Canada (*Rapporteur*)

Professor G. Pahlke, Director, Federal Health Institute, Berlin

Dr L. Schinetti, Società del Plasmon S.p.A., Milan, Italy

Dr S. J. Yaffe, Professor of Pediatrics, State University of New York, Buffalo, N.Y., USA

Secretariat :

Dr C. Agthe, Chemical Carcinogenesis Unit, International Agency for Research on Cancer, Lyon, France

Dr E. M. DeMaeyer, Medical Officer, Nutrition, WHO, Geneva, Switzerland

Dr G. D. Kapsiotis, Senior Officer, Nutrition Division, FAO, Rome, Italy

Dr L. G. Ladomery, Food Standards Officer, FAO/WHO Food Standards Programme, FAO, Rome, Italy

Dr F. C. Lu, Chief, Food Additives, WHO, Geneva, Switzerland (*Joint Secretary*)

Mr R. K. Malik, Food Policy and Food Science Service, Nutrition Division, FAO, Rome, Italy (*Joint Secretary*)

1. INTRODUCTION

Following a recommendation of the First Joint FAO/WHO Conference on Food Additives [1] a series of annual meetings of the Joint FAO/WHO Expert Committee on Food Additives have been convened. Since the sixth meeting, the Expert Committee has been given the task of evaluating the safety of individual food additives following the principles and procedures laid down earlier. However, in the case of additives in baby food special considerations are needed because the detoxicating mechanisms that are effective in the more mature individual may be ineffective in the baby. Baby foods should be prepared without food additives whenever possible. Where the use of a food additive becomes necessary in baby foods, great caution should be exercised regarding both the choice of additive and its level of use.

A WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives, which met in July 1966, considered that " ... there are circumstances in which the benefit to the baby arising from the inclusion of some additive, for example a preservative, in its diet may greatly outweigh any possible hazard to health ". With reference to unintentional contaminants, it stressed that " the diet of babies is likely, under present-day conditions, to contain traces of pesticide and other residues ".[2]

Following a recommendation made in the tenth report of the Joint FAO/WHO Expert Committee on Food Additives,[3] the present Meeting was convened by FAO and WHO to study the special problems arising from exposure of infants and young children to food additives. It was opened by Dr M. Autret, Director, Nutrition Division, FAO, on behalf of the Directors-General of FAO and WHO.

2. GENERAL CONSIDERATIONS

2.1 Scope

The task of the Meeting was confined to the consideration of additives that are either not nutrients or not added to baby foods primarily for nutritional purposes. In addition to intentional additives, the Meeting also considered pesticide residues and other contaminants in baby foods. A

---

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1956, No. 107.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348, p. 11.

[3] *FAO Nutrition Meetings Report Series*, 1967, No. 43 ; *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 373.

baby food was defined by the Meeting as any food that is directly or by implication represented as being intended for feeding to infants and young children.

2.2 Principles for the use of intentional food additives

2.2.1 *Food for children up to 12 weeks*

On developmental grounds an arbitrary distinction may be made between children aged less than 12 weeks and older children. It is likely that the detoxicating mechanisms, the permeability of certain tissues, and other protective mechanisms of the infant aged up to 12 weeks may not have developed to a point where they are able to cope with substances that present no problem to the adult. There is little evidence regarding the age of maturation of detoxicating mechanisms, particularly as regards individual variability. However, it may be assumed that by the end of the twelfth week most of the necessary protective mechanisms have developed. Few additives have been investigated in relation to their effects on very young children. It is therefore prudent that foods intended for infants under 12 weeks should contain no additives at all. Such food would include infant formulas (and other milk-based preparations), cereal-based baby foods, and " strained " foods and fruit juices. Certain cereal-based baby foods, " strained " foods, and " junior " foods that are intended for older babies may contain additives and should be adequately labelled to ensure that they are not consumed by infants under 12 weeks.

It was accepted by the Meeting that in practice there may be certain exceptions on technological grounds to the exclusion of food additives from food for infants under 12 weeks. The use of food additives may be justified, for example, to increase shelf life, to ensure adequate sterilization by promoting homogenization, or to maintain consistency and texture in order to ensure safe and acceptable use. However, appeal to the eye or organoleptic acceptability to the mother, as opposed to the infant, does not constitute justification. The Meeting also accepts that in some countries with special difficulties—for example, of storage or supply—additives not otherwise acceptable may confer advantages that outweigh their potential hazards.

2.2.2 *Foods for children over 12 weeks*

The availability of " junior " and " strained " foods confers an advantage in that the infant may be given a diet more varied and therefore often nutritionally more satisfactory than it would otherwise receive.

Although after 12 weeks detoxicating and other protective mechanisms may be adequately developed, it must be remembered that, in terms of caloric intake per kilogram of body weight, young children consume up

to three times as much as adults. This factor needs to be borne in mind when considering levels of additives in foods designed for young children.

2.2.3 *Standards of safety of additives*

The Meeting considered that, particularly for infants under 12 weeks, toxicological investigations should be more extensive and include evidence of safety to young animals. Technological justification should be given less weight than in the case of foods intended for older babies or adults. Details are given in section 3.

2.3 Pesticides and contaminants

The above considerations on intentional additives also apply to pesticide residues and other contaminants. Ideally, pesticide residues and other contaminants, including environmental pollutants, should be absent, but it is recognized that no food, not even that intended for babies aged less than 12 weeks, can be expected to be completely free from such substances. There are situations where some contamination cannot be avoided, for example from carry-over or from equipment.

The establishment of acceptable residue levels of pesticides or other contaminants likely to be present in milk and cereals for infant foods should be based on toxicological evaluation in very young animals. Until such results are available, baby foods should be manufactured from raw materials selected and processed in such a way that only minimal residues are present in the final product.

2.4 Other general considerations

The Meeting expressed concern that in addition to foods specifically intended for babies other foods mainly intended for adults may be eaten by children. In particular, processed dairy products, bread, and biscuits, all of which may contain additives, are often fed to babies instead of cereal-based foods specifically prepared for infants. The Meeting draws the attention of the appropriate expert bodies of FAO and WHO to this problem.

3. TECHNOLOGICAL CONSIDERATIONS

The Meeting was aware that any approval of additives at present would be of a temporary nature until the requirements for toxicological testing as laid down in section 4.2 have been met. The raw materials should be selected so as to contain a minimum of contaminants, as discussed in section 2.3. On individual groups of additives the following conclusions were reached.

3.1 Chemical preservatives

These should not be used in baby food except in special circumstances as specified in paragraph 2.2.1. Raw materials treated with nitrites or nitrates should be avoided.

3.2 Food colours

In keeping with the principles laid down in section 2, these should not be used for the express purpose of colouring.

3.3 Emulsifiers, stabilizers, and thickeners

These are necessary for a variety of baby foods. Experience from some countries suggests that starch is suitable for thickening and stabilizing purposes for infant formulas.[1] Lecithin, monoglycerides and diglycerides appear to be suitable for emulsification purposes. Gelatin and sodium and calcium caseinates, which are also used, are not regarded as additives.

3.4 Flavouring agents and flavour enhancers

These are not necessary in infant formulas. However, babies over 12 weeks of age may be capable of distinguishing flavours. All baby foods should be prepared in such a way that their natural flavour is retained, if desired. In certain processes, however, such as sterilization or deaeration, it is not always possible to retain the original flavour. In such cases, the restoration of desirable natural flavours may be justified. For the manufacture of some composite foods, including food ingredients from novel sources, the addition of natural flavours from edible products may also be justified. On the other hand, there appears to be no technological justification for flavour enhancers. Although salt may be regarded as a flavouring agent, sodium and chloride are necessary nutrients in infant formulas. If sodium chloride is added to other classes of baby foods for taste purposes, it should be limited to 0.25% by weight of the product to be consumed (see also section 5.5).

3.5 Antioxidants

Some antioxidants are needed for a variety of baby foods. Experience shows that, under favourable climatic conditions, a normally acceptable

---

[1] See the fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives, *FAO Nutrition Meetings Report Series*, 1971, No. 48 ; *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 462.

shelf-life can be maintained for some foods solely by the use of such naturally occurring antioxidants as tocopherols and ascorbic acid, or their appropriate esters. Packaging under nitrogen or carbon dioxide may be required in certain cases.

3.6 Buffers and pH regulators

A variety of suitable chemical compounds are available.

4. TOXICOLOGICAL CONSIDERATIONS

4.1 Vulnerability of very young infants

Although breast feeding is recognized to be the commonest form in which the newborn infant receives its food supply in the early weeks of life, comparatively few babies are still breast fed at 6 weeks of age in the more highly developed countries. Many infants have by then experienced contact with infant formulas as substitutes for milk, and some may receive their entire food supply in that form from the day of birth. Furthermore, cereal-based infant foods and other classes of infant foods, such as strained foods and supplementary infant foods, may have been introduced at various ages and provide an appreciable proportion of the caloric intake for infants between 3 and 6 months. Thereafter, the child is increasingly fed from the family meals, while cereal consumption tends to wane and canned strained foods and " junior " convenience foods often become more important constituents of the diet.

Very young infants are especially vulnerable to foreign chemicals because the mechanisms that provide protection against these substances are absent or not fully developed. Although the evidence for this derives mainly from studies with drugs rather than with food additives, it is likely that such very young infants are less efficient than older children in metabolizing some food additives and may therefore accumulate them to excessive levels. If this occurs at a time when sensitivity to toxic effects is critical because of the delicately balanced growth and differentiation processes, there may be deleterious consequences that may not appear until much later in the child's development. Very young infants may also differ from older children in relation to physiological barriers protecting sensitive tissues, such as the blood-brain barrier or the protective barriers for retinal or lens tissue.

The known differences between the very young infant and the older child include the following : low gastric acid secretion, different biochemical capabilities of the gastrointestinal tract, inability to digest and absorb certain

substances, deficiency in methaemaglobin reductase systems, diminished renal excretory processes at both glomerular and tubular sites.

4.2 Toxicological testing

After reviewing the guidance given in the various FAO/WHO publications for the toxicological testing of intentional and unintentional food additives, it was considered that modification of some of the recommended procedures was necessary. Before a food additive is regarded as safe for use in food intended for infants up to 12 weeks of age, the toxicological studies should be extended to include animals in the corresponding period of life. There is, however, a lack of precise information relating to this developmental period in both animals and the human infant. It is difficult to recommend precise toxicological testing procedures until more basic research has been undertaken. There are also difficulties in selecting the appropriate species. In these circumstances short-term studies should be conducted in several species and should include the oral administration of the additive under test, at suitable dose levels, to newly born animals up to and including the end of the weaning period. This type of exposure is necessary in order to discover whether the existing species variation seriously affects the handling of the food additive by the test animals and to compensate partly for the serious lack of knowledge in this area both in animals and men.

When lifespan studies and multigeneration studies are carried out, they should be extended to include the oral administration of the food additive at suitable dose levels to a proportion of animals from the day of birth throughout the pre-weaning period. These animals should then be observed for the required period as laid down for the usual lifespan and multigeneration tests. Multigeneration tests should be carried out for at least 3 generations. If there are any indications of heritable mutagenic effects these tests should be followed by specific studies. Some members of the filial generations should be exposed to the additive for their lifespan. The enhanced sensitivity of the newborn of several species to certain carcinogenic agents is particularly relevant in this regard. It is also necessary to place greater emphasis in future on observations of the behavioural developments of animals used for toxicological testing, particularly in long-term studies. Because of uncertainties still existing in this field, the accumulation of experimental information is of paramount importance for the evaluation of the results in terms of human health hazard.

The Meeting emphasized the importance of the recommendations made by the WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives with regard to the desirability of supplementing animal studies on additives by investigations in man. It considered that this applies specially to additives to be used in infant foods.

5. SPECIFIC FOOD ADDITIVES

Certain food additives in respect of which specific queries had been raised are considered below. These considerations do not imply that any greater significance should be accorded to these additives than to others not specially considered.

5.1 Modified starches

Certain modified starches were stated in the thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives [1] to be equivalent to normal products of digestion. These included starches treated by physical means, acid-treated starches, enzyme-treated starches, and alkali-treated starches. The Meeting considered that there could be no objection to their being used as additives in foods for infants beyond 12 weeks of age.

5.2 Lactic acid

This food additive was re-evaluated at the thirteenth meeting of the Joint FAO/WHO Expert Committee [1] and the previous restriction on the use of the D-enantiomorph in the diet of very young infants was upheld. The evidence for this decision appears in the appropriate monograph.[2] No additional evidence has come forward to indicate a need to change the opinion expressed in the ninth report of the Joint FAO/WHO Expert Committee that only L-lactic acid be used in the preparation of infant foods.[3]

5.3 Nitrates and nitrites

There are wide variations in the occurrence of nitrates and nitrites in vegetables and certain prepared meat products. These substances are of special importance because of the extreme sensitivity of the human infant to the induction of methaemoglobinaemia. Because the mechanism of methaemoglobin reduction is poorly developed and because of the increased liability of fetal erythrocytes to methaemoglobin formation, during the first 2–3 months of life there is a greater risk of adverse reactions from absorbed nitrite. This nitrite is derived essentially from bacterial

---

[1] *FAO Nutrition Meetings Report Series*, 1970, No. 46 ; *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445.

[2] *FAO Nutrition Meetings Report Series*, No. 40 A, B, C ; WHO/Food Add/67.29.

[3] *FAO Nutrition Meetings Report Series*, 1966, No. 40 ; *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 339.

activity in the gastrointestinal tract, a process that occurs particularly readily in the very young infant because of the inadequacy of acid production in the stomach. It is therefore recommended that every effort should be made to reduce to a minimum the total nitrate and nitrite intake of infants. It is also necessary to consider the possibility of nitrosamine formation *in vivo* following simultaneous ingestion of nitrite and amine-containing foods. Almost all the nitrosamines hitherto examined in animal tests have been shown to possess a carcinogenic potential. In view of the particular sensitivity of the young infant to these toxic agents this is a further reason for restricting nitrate and nitrite intake to a minimum. It has also been shown that the presence of nitrate in water used in the processing of canned food may lead to excessive concentrations of tin with possible consequent deleterious effects.

5.4 Phosphates and calcium

The level of phosphates in the diet must be considered in relation to the calcium intake. There is a wide latitude for dietary variations in calcium content without significant toxicological effects and the calcium contributed from food additives is unlikely to make a substantial impact on total intake. The Meeting agreed that the calcium-phosphorus ratio in a baby food should not be less than 1 : 1.2. This provision should be made to apply at least to infant formulas and cereal-based baby foods.

The toxicological aspects of phosphates as food additives were reviewed in the seventh report of the Joint FAO/WHO Expert Committee.[1] The contribution to the phosphate load from the carry-over of phosphates used as additives in certain ingredients of infant formulas should be allowed for when calculating the general adjustment of the calcium-phosphorus ratio in infant foods.

5.5 Sodium compounds

The investigation of various infant foods and infant formulas has revealed a considerable variation in the total amounts of sodium, depending largely on the combination of the foods. There is some evidence, both from animal experiments and from epidemiological studies in man, of a correlation between sodium intake and the appearance of hypertension. Restriction in the sodium content by limiting the amount of salt to be added to infant food is therefore recommended (see section 3).

---

[1] *FAO Nutrition Meetings Report Series*, 1964, No. 35 ; *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 281.

6. RECOMMENDATIONS

(1) Fundamental research should be carried out on methods of toxicological testing of substances intended for use as additives in infant foods, special attention being given to the problem of differences in pre-weaning development between the test animals and man, thus permitting more reliable predictions of toxicity to the human infant.

(2) WHO should promote toxicological studies of those additives, the use of which is essential in baby foods.

(3) As new pertinent information becomes available, the safety of additives in all foods taken by infants should be reassessed.

(4) A more thorough investigation should be made of the standards and criteria used for assessing the technological justification for the use of additives used in baby food.

(5) In evaluating the safety of food additives, the possibility should be borne in mind that certain classes of foods containing additives may be consumed by infants as well as by adults.

Annex 4

EVALUATION OF COMMERCIAL ENZYMES

	Evaluation [a]	*Monograph prepared*	*Specification prepared*
I. *Animal-derived enzyme preparations*			
1. Catalase (bovine liver)	Not limited	No	Yes
2. Lipase, animal	Not limited	No	Yes
3. Pepsin	Not limited	No	Yes
4. Rennet	Not limited	No	Yes
5. Rennet, bovine	Not limited	No	Yes
6. Trypsin	Not limited	No	Yes
II. *Plant-derived enzyme preparations*			
1. Bromelain	Not limited	No	Yes
2. Ficin	Decision postponed	No	Yes [c]
3. Malt, carbohydrases	Not limited	No	Yes
4. Papain	Not limited	No	Yes
III. *Microbial enzyme preparations*			
1. *Aspergillus niger* varieties			
(*a*) Carbohydrase	Not limited [b]	Yes	Yes [c]
(*b*) Glucose oxidase	Not limited [b]	Yes	Yes [c]
(*c*) Catalase	Decision postponed	No	Yes [c]
2. *Aspergillus oryzae* varieties			
(*a*) Carbohydrases	Decision postponed	No	Yes [c]
(*b*) Protease	Decision postponed	No	Yes [c]
(*c*) Lipase	Decision postponed	No	Yes [c]
3. *Bacillus cereus*			
Microbial rennet	Decision postponed	No	Yes [c]

[a] Not limited = use limited by good manufacturing practice.
[b] Temporary evaluation.
[c] Tentative specification.

	Evaluation [a]	*Monograph prepared*	*Specification prepared*
4. *Bacillus subtilis* varieties			
Carbohydrases and proteases, mixed	Not limited	Yes	Yes
5. *Endothia parasitica*			
Microbial rennet	Not limited [b]	Yes	Yes [c]
6. *Mucor miehei*			
Microbial rennet	Not limited [b]	Yes	Yes [c]
7. *Mucor pusillus*			
Microbial rennet	Not limited [b]	Yes	Yes [c]
8. *Micrococcus lysodeikticus*			
Catalase	Decision postponed	No	Yes [c]
9. *Rhizopus oryzae*			
Carbohydrase	Not limited	No	Yes
10. *Saccharomyces* sp.			
Carbohydrase	Not limited	No	Yes
11. *Miscellaneous*			
Irpex lacteus (microbial rennet)	Decision postponed	No	No
Penicillium amagasakiense (microbial glucose oxidase)	Decision postponed	No	No
Arthrobacter (microbial carbohydrase)	Decision postponed	No	No

[a] Not limited = use limited by good manufacturing practice.
[b] Temporary evaluation.
[c] Tentative specification.

Annex 5

EVALUATION OF MODIFIED STARCHES [a]

	Evaluation [b]	*Monograph prepared*	*Specification prepared*
Oxidized starches	Not limited	Yes	Yes
Starch acetate	Not limited	Yes	Yes
Hydroxypropyl starch	Not limited	Yes	Yes
Distarch phosphate	Not limited	Yes	Yes
Acetylated distarch phosphate	Not limited	Yes	Yes
Distarch glycerol	Not limited	Yes	Yes
Acetylated distarch glycerol	Not limited	Yes	Yes
Hydroxypropyl distarch glycerol	Not limited	Yes	Yes
Acetylated distarch adipate	Not limited	Yes	Yes
Phosphated distarch phosphate	Not limited	Yes	Yes
Hydroxypropyl distarch phosphate	Not acceptable	Yes	Yes [c]

[a] For evaluations of other modified starches, see Annex 4 to thirteenth report, FAO *Nutrition Meetings Report Series*, 1970, No. 46 ; *Wld Hlth Org. techn. Rep. Ser.*, 1970, No. 445.

[b] Not limited = use limited by good manufacturing practice ; temporary acceptances.

[c] Tentative specification.

Annex 6

EVALUATION OF MISCELLANEOUS FOOD ADDITIVES

Substance	*Acceptable daily intake for man* [a] *(mg/kg body-weight)*	*Monograph prepared*	*Specification prepared*
Caramel colours			
(*a*) made by ammonia processes	0-100 [b, c]	Yes	Yes [f]
(*b*) made by other processes	Not limited [d]	No	Yes
Choline salts	Not limited [d]	No	No
Esters of glycerol and thermally oxidized soya bean fatty acid	0-75 [b]	Yes	Yes [f]
Thermally oxidized soya bean oil	No ADI	No	Yes [g]
Hexamethylenetetramine	0-5 [b]	Yes	Yes
Microcrystalline cellulose	Not limited [d]	Yes	Yes
Propylene glycol alginate	0-25	Yes	Yes
Stearoyl lactylate, calcium and sodium salts	0-20	Yes	Yes
Tin and stannous chloride	No ADI [e]	Yes	Yes

[a] Unconditional unless otherwise stated.

[b] Temporary ADI.

[c] The level of 4-methylimidazole should not exceed 200 mg/kg, based on a product having a colour intensity of 20 000 EBC units (see footnote on p. 16).

[d] Use limited by good manufacturing practice.

[e] See section 5.3.9, p. 19.

[f] Tentative specification.

[g] See section 5.3.5, p. 18.

WORLD HEALTH ORGANIZATION

TECHNICAL REPORT SERIES

Recent reports :

No.		Price p	Price $	Price Sw. fr.
443	(1970) **Insecticide Resistance and Vector Control** Seventeenth Report of the WHO Expert Committee on Insecticides (279 pages)	£1.20	4.00	12.—
444	(1970) **WHO Expert Committee on Biological Standardization** Twenty-second Report (134 pages)	60	2.00	6.—
445	(1970) **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 of the Joint FAO/WHO Expert Committee on Food Additives (36 pages)	30	1.00	3.—
446	(1970) **Clinical Pharmacology: Scope, Organization, Training** Report of a WHO Study Group (21 pages)	20	0.60	2.—
447	(1970) **WHO Expert Committee on Plague** Fourth Report (25 pages)	20	0.60	2.—
448	(1970) **Factors Regulating the Immune Response** Report of a WHO Scientific Group (88 pages)	50	1.75	5.—
449	(1970) **Dental Health Education** Report of a WHO Expert Committee (28 pages)	20	0.60	2.—
450	(1970) **Biological Research in Schizophrenia** Report of a WHO Scientific Group (35 pages)	30	1.00	3.—
451	(1970) **Wholesomeness of Irradiated Food with Special Reference to Wheat, Potatoes and Onions** Report of a Joint FAO/IAEA/WHO Expert Committee (44 pages)	30	1.00	3.—
452	(1970) **Requirements of Ascorbic Acid, Vitamin D, Vitamin B_{12}, Folate and Iron** Report of a Joint FAO/WHO Expert Group (75 pages)	40	1.25	4.—
453	(1970) **Joint FAO/WHO Expert Committee on Milk Hygiene** Third Report (82 pages)	40	1.25	4.—
454	(1970) **Multipurpose Serological Surveys and Serum Reference Banks** Report of a WHO Scientific Group (95 pages)	50	1.75	5.—
455	(1970) **Treponematoses Research** Report of a WHO Scientific Group (91 pages)	50	1.75	5.—
456	(1970) **Training in National Health Planning** Report of a WHO Scientific Group (59 pages)	40	1.25	4.—
457	(1970) **The Prevention of Perinatal Mortality and Morbidity** Report of a WHO Expert Committee (60 pages)	40	1.25	4.—
458	(1970) **Pesticide Residues in Food** Report of the 1969 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Group on Pesticide Residues (43 pages)	30	1.00	3.—
459	(1970) **WHO Expert Committee on Leprosy** Fourth Report (31 pages)	30	1.00	3.—
460	(1970) **WHO Expert Committee on Drug Dependence** Eighteenth Report (45 pages)	30	1.00	3.—