

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

Evaluation of certain food additives

Twenty-first Report of the
Joint FAO/WHO Expert Committee on
Food Additives



World Health Organization
Technical Report Series
617



World Health Organization Geneva 1978

Monographs containing summaries of relevant data and toxicological evaluations are issued separately by WHO under the title :

Toxicological evaluation of certain food additives
WHO Food Additives Series No. 12

Specifications are issued separately by FAO under the title :

Specifications for the identity and purity of certain food additives
FAO Nutrition Meetings Report Series No. 57

ISBN 92 4 120617 9

© World Health Organization 1978

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. For rights of reproduction or translation of WHO publications, in part or *in toto*, application should be made to the Office of Publications, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

PRINTED IN SWITZERLAND

CONTENTS

	Page
1. Introduction	5
2. General considerations	5
2.1 Modification of agenda	5
2.2 General principles for the evaluation of food colours	6
2.3 Safety aspects of enzyme preparations used in food processing	8
2.4 Problems of exposure of infants and children to contaminants in food	9
2.5 Microbiological criteria for food additives	12
2.6 Supply of information	12
3. Comments and evaluations	13
3.1 Acids and salts	13
3.2 Antioxidants	14
3.3 Food colours	15
3.4 Sweeteners	23
3.5 Thickening agents	26
3.6 Miscellaneous	27
4. Review of specifications	28
5. Future work	31
6. Recommendations to FAO and WHO	31
Annex 1. Reports and other documents resulting from previous meetings of the Joint FAO/ WHO Expert Committee on Food Additives	33
Annex 2. Acceptable daily intakes and information on specifications	37
Annex 3. Further toxicological studies and information required	40

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva, 18-27 April 1977

Members invited by FAO

- Mr D. F. Dodgen, Director, Food Chemicals Codex, National Academy of Sciences, Washington, DC, USA
- Dr H. Eich, Besigheim, Federal Republic of Germany
- Dr M. Fujita, Chief, Laboratory of Hygienic Chemistry, National Institute of Public Health, Tokyo, Japan
- Mr A. W. Hubbard, Head, Food Science Division, Ministry of Agriculture, Fisheries and Food, London, England (*Vice-Chairman*)
- Dr C. F. Jelinek, Deputy Associate Director for Technology, Bureau of Foods, Food and Drug Administration, Washington, DC, USA
- Dr W. Kroenert, Head, Food Chemistry Division, Federal Office of Public Health, Berlin (West)

Members invited by WHO

- Dr B. Briski, Chief, Division for Laboratory Food and Food Additives Analysis, Institute of Public Health, Zagreb, Yugoslavia
- Dr G. J. van Esch, Director, Toxicology and Foodstuffs, National Institute of Public Health, Bilthoven, Netherlands
- Dr E. Poulsen, Director, National Food Institute, Institute of Toxicology, Søborg, Denmark (*Chairman*)
- Dr P. Shubik, Director, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE, USA
- Professor R. Truhaut, Director, Toxicological Research Centre, René Descartes University, Paris, France
- Professor G. Zbinden, Institute of Toxicology, Federal Institute of Technology and University of Zürich, Switzerland (*Rapporteur*)

Observer invited by FAO

- Dr G. F. Wilmink, Chairman, Codex Committee on Food Additives, c/o Ministry of Agriculture and Fisheries, The Hague, Netherlands

Secretariat

- Dr C. Agthe, Chief, Food Additives, WHO, Geneva, Switzerland (*Joint Secretary*)
- Dr H. Blumenthal, Acting Director, Division of Toxicology, Bureau of Foods, Food and Drug Administration, Washington, DC, USA (*WHO Temporary Adviser*)
- Dr K. O. Herz, Food Policy and Nutrition Division, FAO, Rome, Italy (*Joint Secretary*)
- Dr L. G. Lodomery, Food Policy and Nutrition Division, FAO, Rome, Italy
- Dr I. C. Munro, Chief, Toxicology Research Division, Ottawa, Canada (*WHO Temporary Adviser*)
- Dr G. Vettorazzi, Scientist, Food Additives, WHO, Geneva, Switzerland

EVALUATION OF CERTAIN FOOD ADDITIVES

Twenty-first Report of the Joint FAO/WHO Expert Committee on Food Additives

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 18 to 27 April 1977. The meeting was opened by Dr T. Lambo, Deputy Director-General, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization. Dr Lambo said that the previous recommendations made by the Committee had been of great assistance to Member States and to the Joint FAO/WHO Codex Alimentarius Commission and had proved to be valid instruments in food control programmes. They had always been eagerly awaited by industry and by the health and food regulatory authorities in all countries. In view of the recent concern about the artificial sweetener saccharin, he recommended that re-evaluation of that substance should be considered.

1. INTRODUCTION

The tasks before the Expert Committee were: (1) to prepare specifications and carry out the toxicological evaluation of certain food additives; (2) to review recent findings of toxicological studies of certain food additives; (3) to revise the specifications for certain food additives; (4) to undertake the toxicological re-evaluation of certain food additives; (5) to consider the safety aspects of enzyme preparations used in food processing; (6) to examine the problem of exposure of infants and children to contaminants in food; and (7) to give further general consideration to the principles and procedures of evaluation of food additives and contaminants.

2. GENERAL CONSIDERATIONS

2.1 Modification of agenda

In view of public concern about artificial sweeteners and the many questions by various governments and the public emanating from recent regulatory actions on saccharin by some Member States, the Secretariat proposed a discussion of

saccharin. In addition, a member of the Committee suggested that cyclamates also be considered. The Committee agreed to put the two artificial sweeteners on the agenda.

It was also agreed to delete from the agenda amylase (*Bacillus licheniformis*) and glucose isomerase (*Bacillus coagulans*) because those food enzymes should be considered together with other, currently more widely used enzymes of the same type from different microorganisms.

2.2 General principles for the evaluation of food colours

Natural colours

In the eighth report of the Committee¹ the general principles governing the establishment of specifications for the identity and purity of food colours were spelled out in detail. At its present meeting the Committee reconsidered the question of natural colours and reaffirmed the statement on specifications contained in the eighth report, which read as follows:

Natural colours have been used in food over a long period of time and have been accepted for such use without supporting toxicological evidence in much the same manner as vegetables and cereal products. In considering such food colours, the Committee was faced with serious problems owing to the lack of published information relating to adequate identification and chemical composition. It was noted that natural colours may be available in different forms; in the case of botanical products both the powdered plant and the extract of the powdered plant have been used as colouring agents for many years. Also, because of differences in soil, climatic conditions, age of plant, and time of harvest, the nature and proportions of the coloured and other components of the same species of the same plant may vary widely. Such products may contain a large percentage of substances which have not been defined. Morphological and histological examination of such botanicals has been used for a long time for the identification and quality evaluation of these materials, but in the opinion of the Committee the information obtained in this way is inadequate.

The present Committee expressed its concern over the continued lack of detailed information on the composition of all types of natural colours and reaffirmed the position of previous Committees that naturalness *per se* does not assure safety.

Some of the natural colours are marketed as the dried natural product. Examples are saffron, turmeric, paprika, and dried tagetes meal. In general these products are not well characterized. Furthermore, insufficient information is available concerning their composition, purity, and use levels. Finally, information about microbiological contamination is necessary.

Natural colours are also sold as extracts. Examples of this type are cochineal extract, tagetes extract, and the anthocyanin colour in grapeskin extract. The gaps in information concerning natural colour extracts are essentially the same as those for the dried natural products.

¹ See Annex 1, reference 8.

Oleoresins are another type of natural food colour. They are obtained by extraction of the colouring principle from the natural product and evaporation of the solvent. Examples of such products are turmeric oleoresin, paprika oleoresin, and corn endosperm oil. The same uncertainties pertain to this type of colour as to the dried natural colours. In addition, information is lacking concerning the composition of products obtained by extraction of the same natural product with different solvents.

When a food colour is isolated from natural sources, information concerning the composition of the colour product is generally deficient as compared to that available for synthetic colours. In addition, microbiological specifications are required for the colour isolated from natural sources.

The Committee stressed that the lack of assurance of reproducible composition of natural food colour products makes their toxicological evaluation a difficult task.

For toxicological evaluation, natural colours should be considered as falling within three main groups.

(1) A colour isolated in a chemically unmodified form from a recognized foodstuff and used in the foodstuff from which it is extracted at levels normally found in that food. This product could be accepted in the same manner as the food itself with no requirement for toxicological data.

(2) A colour isolated in a chemically unmodified form from a recognized foodstuff but used at levels in excess of those normally found in that food or used in foods other than that from which it is extracted. This product might require the toxicological data usually demanded to assess the toxicity of synthetic colours.

(3) A colour isolated from a food source and chemically modified during its production or a natural colour isolated from a non-food source. These products would also require a toxicological evaluation similar to that carried out for a synthetic colour.

The Committee recognized that natural colours may be reproduced by chemical synthesis but noted that "nature-identical" colours produced by chemical synthesis may contain impurities warranting toxicological evaluation similar to that required for a synthetically produced food colour.

Synthetic colours

The Committee concluded that the toxicological evaluation of synthetic colours would require the following minimum data:

(1) Metabolism studies in several species, preferably including man. These studies should include absorption, distribution, biotransformation, and elimination, and an attempt should be made to identify the metabolic products in each of these steps.

- (2) Short-term feeding studies in a non-rodent mammalian species.
- (3) Multigeneration reproduction/teratogenicity studies.
- (4) Long-term carcinogenicity/toxicity studies in two species.

Analytical developments

The Committee was aware of recent advances in analytical chemistry (particularly development of high-pressure liquid chromatography) that would enable the chemical composition of food colours to be determined more precisely. Together with technological advances in the manufacture of food colours, this new ability would permit the promulgation of more meaningful specifications for purity and levels of undesirable substances, wherever such specifications were desired.

2.3 Safety aspects of enzyme preparations used in food processing

The problems of safety of enzyme preparations in food processing were discussed at the fifteenth and eighteenth meetings of the Expert Committee,¹ which, in its deliberations regarding approaches to the evaluation of safety of enzymes, prepared a general classification of these products based mainly on sources and preparation procedures. At its present meeting, the Committee reaffirmed those proposals.

The use of enzymes in food is increasing both as processing aids and as additives intended to remain in food products. They are obtained either from animal or plant tissues or from cultures of microorganisms. Only in exceptional cases are these enzymes used as crystallized, pure substances or in highly purified form. In general they are obtained from extracts or fermentation broths and partially purified. From the safety point of view the presence of potentially harmful contaminants and by-products is of concern. The nature and levels of these substances may vary depending on the sources selected for preparation of the enzyme, the culture and the extraction procedures. Moreover, when microorganisms are used for the production of enzymes, mutations might occur that could lead to the emergence of new, potentially toxic products. In its eighteenth report the Committee did not consider this to be a serious problem, but it now felt that chemical and microbiological specifications and the biological control of strains of microorganisms used to produce these food enzymes are of the utmost importance in assuring the safety of these materials.

The increasing use of immobilized enzymes calls for the evaluation of the immobilizing substrates as well as of the immobilizing techniques.

Enzyme preparations used in food may be grouped into five major classes.

¹ See Annex 1, references 26 and 34.

(1) Enzymes obtained from edible tissues of animals commonly used as foods. These are regarded as foods and consequently considered acceptable provided satisfactory chemical and microbiological specifications can be established.

(2) Enzymes obtained from edible portions of plants. These are also regarded as foods and consequently considered acceptable provided that satisfactory chemical and microbiological specifications can be established.

(3) Enzymes derived from microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods. These products are regarded as foods and consequently acceptable provided satisfactory chemical and microbiological specifications can be established.

(4) Enzymes derived from nonpathogenic microorganisms commonly found as contaminants of foods. These materials are not considered as foods. The Committee considers it necessary to establish chemical and microbiological specifications and to conduct short-term toxicity experiments to ensure the absence of toxicity. Each preparation must be evaluated individually and an ADI must be established.

(5) Enzymes derived from microorganisms that are less well known. These materials also require chemical and microbiological specifications and more extensive toxicological studies, including a long-term study in a rodent species.

2.4 Problems of exposure of infants and children to contaminants in food

The problem of the exposure of infants and children to contaminants in foods was discussed by the Committee, as recommended in the twentieth report.¹

In its sixteenth report² the Committee had pointed out that one reason why it was inappropriate to set ADIs for the heavy metals lead, cadmium, and mercury was that the special susceptibility of the fetus, the newborn child, and the young child could not be accurately expressed. It had also pointed out that children can be considered a high-risk group in relation to lead exposure. The Committee had consequently stated that the provisional tolerable weekly intake of 3 mg of lead per person did not apply to infants and children and had recommended that the special problems relating to the exposure of infants and children to contaminants in food should be studied in depth by appropriate expert bodies.

In discussing the toxicological problems arising from the exposure of infants and young children, the Committee, at its present meeting, also considered food additives.

¹ See Annex 1, reference 39.

² See Annex 1, reference 30.

Scientific evidence, much of it derived from animal experiments, and supported by clinical observations, indicates that newborn and very young children are particularly sensitive to the harmful effects of foreign chemicals. Among the reasons for this are the immaturity of enzymatic detoxifying mechanisms, incomplete function of excretory organs, low levels of plasma proteins capable of binding toxic chemicals, and incomplete development of physiological barriers such as the blood-brain barrier. Moreover, there appears to be a general vulnerability of rapidly growing tissues, which is particularly important with regard to the developing central nervous system.

A specific question before the Committee was whether or not the ADIs allocated for food additives can be applied to all age classes including the newborn, infants, and young children. Previous meetings of the Expert Committee have been aware of this problem. A special meeting was held in 1971 and the results were published as an annex to the fifteenth report. As a general conclusion it was stated that foods intended for infants under the age of 12 weeks should (with certain exceptions) not contain any additives.

However, it is unavoidable that older infants will often consume foods not specially formulated for their age group. It was felt that the safety factors used for the determination of ADIs provide for individual variations in metabolism and sensitivities to foreign chemicals. Thus, for most food additives, the ADIs allocated are applicable to all children older than 12 weeks.

In assessing food additive safety, the question of potential special hazards for the newborn and infants should be kept in mind.

It was pointed out that many toxic substances may pass into and sometimes concentrate in mothers' milk—e.g., polychlorinated biphenyls (PCBs), chlorinated hydrocarbon pesticides, and mycotoxins. In many countries mothers' milk constitutes a substantial part of the infant diet not only in the immediate postnatal period but also for many months thereafter.

It should also be remembered that in some countries many mothers do not nurse their children. These children are therefore exposed to any chemicals that may be present in their food. Thus, toxicological and metabolic studies of food additives should always include investigations that permit the evaluation of safety for the newborn and the infant.

In discussing the physiological factors that may explain the higher sensitivity of newborn and young infants, it was pointed out that the same factors are of even greater importance for the embryo *in utero*. Hitherto, toxicological studies have been mainly concerned with embryotoxic effects that manifest themselves by fetal lethality and with teratogenic effects recognizable at birth and during the immediate postnatal period. It has become evident in recent years that exposure to toxic

chemicals *in utero* may have effects that cannot be recognized during these developmental stages but appear later in life as tumours, changes in immunological mechanisms, and disturbances of behaviour and neurological functions.

In order to gain more information about the long-term effects of exposure to chemicals *in utero* and in the postnatal period, appropriate toxicological methodology must be developed. In 1966 a WHO Scientific Group discussed the problem of transplacental carcinogenesis as a potential long-term effect.¹ The present Committee considered this an important problem but drew attention to other possible long-term effects such as changes in behaviour and immunological mechanisms. Since the experimental approach to these problems may depend on the chemical, toxicological and pharmacokinetic properties of the compounds, the Committee did not propose a specific toxicological protocol. However, it emphasized that the short- and long-term effects of exposure *in utero* and during the lactation period should be taken into account for food additives and contaminants evaluated by the Committee. This evaluation might include a request for appropriate animal studies.

The Committee felt that information on contaminant intake by infants and young children is still scanty for most countries and substances. However, the available information on levels of contaminants in foods eaten by infants and young children indicates that primary attention should be directed to lead, mercury, cadmium, PCBs, chlorinated hydrocarbon pesticides, and aflatoxins.

Data on these contaminants in mothers' milk are sparse and irregular, and much of the information is not up to date. Such data should be made available or developed by countries in order that the Expert Committee may evaluate the possible hazards posed by the occurrence of these contaminants in this important infant food.

Adequate background information should be assembled and made available to the Expert Committee in order to arrive at proposals of tolerable intakes for young children up to two years of age for lead, mercury, cadmium, and PCBs. This information could be obtained from WHO's nutrition programme, in which the levels of heavy metals in mothers' milk from certain tropical countries are being determined.

The Committee also recognized that there are many variants in infant feeding customs on a worldwide basis and that nutritional deficiencies and imbalances are an important public health problem. Although these aspects of infants' nutrition are not within the Committee's terms of reference, it is important to remember that the harmful effects of chemicals may be enhanced by nutritional deficiencies. Governments and others concerned with this aspect should take urgent steps to provide data for the sound evaluation of the risks posed by contaminants. Special surveys

¹ WHO Technical Report Series, No. 348, 1967 (*Procedures for investigating intentional and unintentional food additives*).

should be carried out to determine levels of contaminants more likely to occur in certain developing countries, e.g., aflatoxins in groundnuts, maize, milk and mothers' milk, chlorinated hydrocarbon pesticides and heavy metals in rice and other staple foods, and mercury in fish.

2.5 Microbiological criteria for food additives

The twentieth meeting of the Committee had reiterated the need for microbiological criteria for certain food additives and had noted that a consistent rationale was required for applying such criteria to specifications. It had recommended that the second *ad hoc* FAO/WHO Expert Consultation on Microbiological Specifications for Foods be asked to develop the required criteria. A background document had been prepared for the consideration of the Expert Consultation held in Geneva early in 1977.

Pertinent sections of the report of the Expert Consultation were available to the Committee at its present meeting. Most food additives were not expected to be among the more important sources of microbial contamination of foods because of the normally rather small amounts used. It was noted that certain pathogens, such as *Salmonella*, should be absent from all food materials.

The Committee reaffirmed that the major reasons for seeking guidance concerning microbiological criteria in connection with a number of specifications were the need to complete the characteristics of purity (and in some cases also of identity) and to add a microbiological index of good practice to complement that given by limits for chemical contaminants. The necessary guidance is still needed.

The Committee dealt with a number of substances for which microbiological specifications would be important and should be provided. These include natural food colours and colour products (such as anthocyanins, cochineal and carmine, paprika and xanthophylls) and natural gums or resins (karaya, tragacanth and xanthan). The Committee had dealt with other substances on previous occasions and had issued specifications that remain tentative for lack of microbiological specifications. Thus the need for criteria and their application in specifications should be met as a matter of some urgency.

2.6 Supply of information

The Committee reaffirmed the need for adequate information for its preparation of specifications and toxicological evaluation. It therefore recommended that Member Governments requesting an evaluation of a food additive, either directly or through the Codex Alimentarius Commission, should supply the necessary information, including the function of the substance and the justification for its use.

3. COMMENTS AND EVALUATIONS

The Committee evaluated a number of food additives for the first time and also re-evaluated some substances that had been considered at previous meetings. Points of interest arising from these evaluations are set out below. The acceptable daily intakes and information on specifications are summarized in Annex 2 and the further data required for certain substances are listed in Annex 3.

3.1 Acids and salts

Adipic acid

Specifications were available to the Committee. Adipic acid is rapidly metabolized or eliminated unchanged. Data were available to show that this compound is neither teratogenic nor mutagenic. In addition, there was available a two-year rat feeding study. A high dose level of adipic acid causes diarrhoea in rats. The no-effect level in this study was 1% of the diet. An ADI for man of 0-5 mg/kg was established on the basis of the free acid. The ADI is applicable to the potassium, sodium, and ammonium salts as well as to the free acid. A monograph was prepared.

Tartaric acid and monosodium tartrate

The existing specifications for L-(+)-tartaric acid were confirmed. Separate specifications were prepared for synthetic DL-tartaric acid.

L-(+)-tartaric acid was considered during the nineteenth meeting.¹ Since that time, new data have become available on the metabolism of monosodium L-(+)-tartrate and monosodium DL-tartrate and the toxicological evaluation was confirmed for these two substances. A new long-term toxicity study in rats on monosodium L-(+)-tartrate was also available. The metabolic studies indicated that both monosodium L-(+)-tartrate and monosodium DL-tartrate were rapidly cleared from most tissues of rats following cessation of dosing at high levels for seven days. However, monosodium DL-tartrate appeared to accumulate in the kidneys to some extent and to produce increased kidney weight whereas the L-(+)-tartrate salt did not. The new long-term feeding study with monosodium L-(+)-tartrate produced no evidence of carcinogenicity. In this study particular attention was given to renal function and pathology, and no adverse effects were noted even at the highest level fed (7.68%). Growth depression was noted at all dose levels, but at the lowest dose level tested (2.56%) this effect was only transient. It was considered that the reduced growth was not a specific toxic effect but was partially due to unpalatability and

¹ See Annex 1, reference 37.

dilution of caloric intake with a largely nonmetabolized compound. There was no evidence of organ toxicity at any dose level. Thus, a no-effect level could be established at 7.68% of the diet, equivalent to 3000 mg/kg/day. An ADI for man for the L-(+)-tartrate monosodium salt could thus be reconfirmed at 0-30 mg/kg of body weight.¹ The Committee was aware that a certain amount of tartrate occurs naturally in foods. Since adequate long-term studies on monosodium DL-tartrate were not available and this form appeared to produce effects on the kidney at high doses whereas monosodium L-(+)-tartrate did not, the Committee could not establish an ADI for monosodium DL-tartrate. A new monograph was prepared.

3.2 Antioxidants

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)

The existing "tentative" specifications were revised. No new toxicological data were available to the Committee on BHA and BHT. However, there is a need to take note of the fact that the ADIs for BHA, BHT, and tertiary butyl hydroquinone should be considered as a "group ADI".

Tertiary butyl hydroquinone (TBHQ)

The existing specifications were completed by adding an identification test for phenols.

At the twentieth meeting of the Committee it had been concluded that the previously required study of the effect on reproduction of mixtures of BHA and/or BHT with propyl gallate, which had been requested in the seventeenth report,¹ was no longer required and that a reproduction study using each of these antioxidants by themselves was sufficient.

In the evaluation by the nineteenth meeting, the requirement for reproduction studies done with mixtures of antioxidants had been carried over from the seventeenth report. In the light of changes expressed in the twentieth report for BHA and BHT, it is consistent to remove from TBHQ the further requirement for such reproduction studies and to change the temporary ADI to a regular ADI.

The ADI for man was set at 0-0.5 mg/kg of body weight on the basis of a dietary no-effect level in the dog of 1600 mg/kg. No monograph was prepared. The Committee concluded that there should be a group ADI with BHA and BHT.

¹ For food additive use only; expressed as L-(+)-tartaric acid.

² See Annex 1, reference 32.

3.3 Food colours

Alkanet and alkanin

The existing specifications were revoked. The Committee was not aware of any use of these substances as food colour additives. As there were no toxicity data available, no ADI could be established and no monograph was prepared.

Allura Red AC

Tentative specifications were prepared.

Allura Red AC was evaluated during the eighteenth meeting and no ADI was allocated at that time. At the present meeting the Committee had some metabolic studies available as new data. These studies demonstrated that the metabolism of this colour was similar to that of other members of the azo dye class. However, as the Committee was aware that previously requested long-term studies in the rat and mouse would be completed within approximately three years, no evaluation was made and no monograph was prepared. The Committee will re-evaluate this colour when the long-term studies become available.

Aluminium (metal)

Specifications were prepared. The Committee evaluated available toxicological data on aluminium metal as a food additive. These data consisted of studies done with several aluminium salts as well as the metal itself. Aluminium is used as a silvering decoration for certain items of confectionery. The use is very limited and was not considered to present a hazard. A monograph was prepared.

Anthocyanins

Earlier general specifications for "anthocyanins" were reviewed. The Committee recognized the complex nature of this class of colouring materials. Anthocyanins occur in many plants, and particularly in fruits. At least six major anthocyanin compounds have been characterized chemically: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (see section 2.2). They occur in nature as the 3-monoglucosides and the 3,5-diglucosides. Information sufficient to prepare tentative specifications was available for only one food colour product, namely anthocyanin colour from grapeskins.

No toxicological data were available for the isolated colorants. No ADI could be established and no monograph was prepared.

Benzyl Violet 4B

No instance of the use of this colour by a manufacturer in any foodstuff was known to the Committee. Specifications were not revised.

There was evidence from the data available to the Committee that this material had carcinogenic activity in two rat feeding studies. The Committee recognized that the carcinogenic response might be due to an active impurity in the material used in these long-term studies. However, pending the isolation and identification of the specific carcinogenic entity in the colour, it was concluded that this material should not be used in foods. A monograph was prepared.

Black 7984

The Committee was unable to revise specifications because of insufficient information. The long-term studies available on this colour were not considered adequate as regards number of animals and histopathological examination. In addition, the reproduction studies lacked sufficient detail to permit evaluation. No ADI could be established pending the availability of studies meeting the requirements outlined for toxicological evaluation of food colours (see section 2.2). A monograph was prepared.

Brown FK

The existing specifications were not revised. In long-term studies in mice this substance produced hepatic nodules and tissue pigmentation. Also it was shown to be mutagenic in *Salmonella typhimurium* 1538 upon metabolic activation. It was pointed out that this colour contained about 50% impurities. Several of these substances have been identified and some data were available on their metabolism in animals. Some of the metabolites are cardiotoxic. The reproduction/teratogenicity studies were inadequate. No ADI could be established. A monograph was prepared.

Capsanthin and capsorubine

These colouring principles of paprika are not currently manufactured products. The available information on dry paprika products used as a food colour was not sufficient to enable the Committee to prepare specifications. Owing to a lack of toxicological data, no evaluation was possible. No monograph was prepared.

Caramel colours (ammonia process; ammonia-sulfite process)

The existing specifications for caramel colour (ammonia process) were found to be ambiguous since they appeared to cover colours manufactured by the ammonia-sulfite process as well. More accurate definition of the products permitted separate specifications to be established for caramel colour (ammonia process) and caramel colour (ammonia-sulfite process).

Since the last evaluation of this substance during the eighteenth meeting, much additional information has become available. This shows that the two types of caramel colour differ in their toxicity. The principal toxic effect of ammoniated caramel is depression of circulating lymphocytes and leucocytes.

Feeding studies with both types of caramel have demonstrated pigment deposition in mesenteric lymph nodes. The Committee considered that this latter effect was a physiological response. Teratology studies with these substances have not demonstrated any adverse effect.

In short-term (10-week) studies with rats fed ammoniated caramel, a no-effect level for lymphocyte depression was not determined. The lowest level fed was 0.5% of the diet. A similar effect was noted in long-term rat studies at 3% of the diet. Lower levels have not been adequately tested in long-term studies. In view of these data the Committee concluded that a no-effect level could not be determined and thus the temporary ADI for non-sulfited ammoniated caramels was revoked.

As far as ammoniated sulfited caramels are concerned, toxicological evaluation is complicated by conflicting results on the effects of the substances on circulating lymphocytes and leucocytes. In addition the chronic toxicity study reported was considered to be inadequate as a result of a poor survival rate.

Although 4-methylimidazole is no longer considered to be a concern and the introduction of chemical specifications had limited its concentration, the Committee decided to maintain the limit for this substance in specifications to indicate good manufacturing practice. An ADI for man of 0–100 mg/kg of body weight was retained for the ammoniated sulfited caramels. This ADI remains in temporary status until data from an adequate chronic study are reviewed.

Further work required for caramel colours (ammonia-sulfite process) will be adequate carcinogenicity/teratogenicity studies. In these studies, particular attention should be given to bone marrow and immune competence. Attempts should be made to identify the component or components responsible for the reported effects. With regard to caramel colours (ammonia process), until the principle causing the reported effects is identified, no subsequent toxicological evaluation will be undertaken. A monograph was prepared.

Carbon black

Two types of source materials can be used to manufacture the carbon blacks that have been used as food colouring agents. Carbon blacks from vegetable materials such as peat differ considerably from products based on hydrocarbons such as natural gas with respect to ash content and other impurities. The content of polynuclear aromatic hydrocarbons (PNAs) depends on the nature of the source material and the carbonizing conditions, especially the temperature and the time at high temperature.

Analytical methods applied in the past have not resolved the question of how strongly and irreversibly PNAs are absorbed on carbon black. Given the increases in sensitivity of some of the methods under study, PNAs will be detected in some extracts of carbon black. Until the levels that can be extracted are known, no limits for PNAs can be indicated. The Committee was aware of the studies on analyti-

cal methodology for PNAs that are being carried out by the Commission on Food Additives of the International Union of Pure and Applied Chemistry and would welcome seeing consideration of the problems related to carbon black included in the Commission's work. Revision of specifications was not considered. No evaluation could be made. No monograph was prepared.

Carthamus

Tentative specifications were prepared by the Committee for both carthamus yellow and carthamus red.

A short-term toxicity study in rats was available for carthamus yellow. However, since data were lacking on long-term studies, reproduction/teratology and metabolism, no ADI could be established. A monograph was prepared.

Chocolate Brown FB

This product is no longer manufactured as a food colour additive. Specifications were not prepared.

Short- and long-term toxicity studies in rats and mice were available. However, Chocolate Brown FB is known to be a complex mixture of dyes, and the Committee was unable to relate the toxicological data to a substance with defined specifications. Therefore no toxicological evaluation could be made. A monograph was prepared.

Chocolate Brown HT

Revised tentative specifications were prepared.

Satisfactory carcinogenicity/toxicity studies in two rodent species were available as well as a short-term study in a non-rodent species. However, data on reproduction and metabolism were not available. Based on a no-effect level of 0.1% in a long-term mouse study, a temporary ADI for man of 0-0.25 mg/kg of body weight was established. Further work required will be multigeneration reproduction/teratology studies and metabolism studies in several species, preferably including man. A monograph was prepared.

Chrysoine

The Committee was unaware of any current source of chrysoine. No revised specifications were prepared.

The limited toxicological data available on this substance were not of sufficient quality nor was there adequate detail to permit an evaluation. A monograph on chrysoine was prepared.

Cochineal, carmine, and carminic acid

The existing tentative specifications for cochineal and carminic acid were reviewed. Cochineal, the dried ground bodies of female *Dactilopus coccus* Costa,

may be in use as a food colour. Cochineal extract, but not carminic acid *per se*, is known to be used as a food colour. Additionally, food colour products known as carmine lakes exist. Sufficient information was available to prepare specifications for aluminium and aluminium-calcium lakes of carminic acid but not for other types of products including the alkali salts of carminic acid. Further information is required on (1) the food colour uses of cochineal, (2) residual methanol, protein, and carminic acid contents of commercial cochineal extract, and (3) methods of production, levels of use, and composition of the extract including impurities of alkali salts of carminic acid.

The only adequate toxicity studies available were a short-term study in rats and teratogenicity studies in rats (carmine) and mice (lithium and sodium carmine). Owing to the lack of other toxicity data no evaluation could be made. No ADI was established. A monograph was prepared.

Fast Red E

Tentative revised specifications were prepared. Long-term studies in both rats and mice have been published for this substance. However, these reports lack data on histopathological assessment and on chemical and biochemical properties. Consequently no evaluation was made. No monograph was prepared.

Fast Yellow AB

No source of this food colour product could be identified. The existing specification was not revised. Limited metabolic data were available on this compound. The published long-term rat-feeding study was deficient in detail and did not permit an adequate assessment of toxicity. Thus no evaluation was made and no monograph was prepared.

Gold

In view of the rare use of gold as a colorant and the lack of knowledge of the exact nature of the gold used on or in foods, no specification was prepared. No data were available on the toxicity of elemental gold. In view of the very small amounts likely to be ingested by an individual, the Committee did not consider that the use of gold would present a hazard. No monograph was prepared.

Lithol Rubine BK (calcium salt)

Tentative specifications were prepared.

The Committee was aware of the existence of multigeneration teratology studies and of a long-term study in the rat. However, as the details of the latter study were not available, it could not be used for evaluation. Thus the toxicity of the compound could not be assessed. No monograph was prepared.

Lycopene

No manufacturer of lycopene as a food colour was known to the Committee. No specifications were prepared.

This substance is a natural constituent of food plant material (see section 2.2). The data available on an unspecified material consisted of a single-generation reproduction study and a short-term study in the rat. These studies were inadequate from the point of view of the number of animals involved and the factors examined. Thus no evaluation could be made. No monograph was prepared.

Orange I

The Committee was not aware that any manufacturer produces this colour for food use. The existing specifications were not revised. The Committee reviewed summaries of short-term studies on this compound in several animal species. All these studies were carried out at dose levels that produced toxic effects. Published summaries of long-term studies in rats, mice, and dogs were also available. However, in none of these studies was a no-effect level established. Thus no ADI could be set. A monograph was prepared.

Orange GGN

No current food additive use was known to the Committee. The existing specifications were not revised. Long-term toxicological studies in the rat have been carried out with this substance, but the published data are lacking in detail and inadequate for toxicological evaluation. A monograph was prepared.

Orange RN

Information was not available to permit the revision of specifications. Metabolites of this colour include aminophenols and aniline. Adverse effects on the haemopoietic system and the production of Heinz bodies were noted in short- and long-term studies in rats and mice. In addition, bile duct proliferation occurred in a dose-related manner in pigs fed this colour. A no-effect level for this response was not established. No ADI was established. A monograph was prepared.

Persian Berries

The Committee was not aware of any food use of this colour. No specifications were prepared. There were no toxicological data available on this material and hence no evaluation could be made. No monograph was prepared.

Ponceau 6R

The Committee was not aware that any manufacturer produces this colour for food use. Specifications were not revised. As no new toxicological data were

available to the Committee since its eighteenth meeting, no new evaluation was made. No new monograph was prepared.

Ponceau SX

The Committee was not aware that any manufacturer produces this colour for food use. Specifications were not revised. Limited metabolic data were available. Adequate long-term studies were available in the rat and mouse. These studies indicated no carcinogenic effects at the highest level fed (5%). In the dog there was a dose-related atrophy of the adrenal zona glomerulosa as well as haemorrhagic lesions and blood-filled mucosal projections into the urinary bladder at all dose levels. The Committee considered that a temporary ADI could not be established until further studies in the dog were undertaken to define the no-effect level. A multigeneration reproduction/teratology study would also be required. A monograph was prepared.

Quercetin and quercitron

The Committee was not aware that any manufacturer produces these colours for food use. The specifications were not revised. These substances were discussed at the eighth and thirteenth meetings of the Committee.¹ No new data were available apart from mutagenic studies with quercetin demonstrating positive effects in two strains of *Salmonella typhimurium*; however, the purity of the material tested was not reported. The available toxicological data were inadequate to evaluate these materials and no ADI could be established. No monograph was prepared.

Red 2G

The existing specifications, which are tentative, were revised to provide for a total dye assay. Metabolic data available to the Committee on this colour demonstrated that major metabolites in the rat and rabbit included aminophenols and aniline. Both short- and long-term toxicity studies in rats and mice demonstrated depression of erythropoiesis and the production of Heinz bodies. The lowest no-effect level was obtained in a short-term rat study when the colour was incorporated into sausage-meat comprising 80% of the diet. In this study the no-effect level was about 25 mg/kg in terms of the whole diet. On that basis a temporary ADI for man was established at 0–0.006 mg/kg of body weight. Further work required is a multigeneration reproduction/teratology study as well as studies on bone marrow to elucidate the toxic effects on erythropoiesis. A monograph was prepared.

Saffron

Crocin and crocetin, the main colouring principles of saffron, are not produced as food colours. Revised tentative specifications were prepared for saffron. No

¹ See Annex 1, references 8 and 19.

toxicological data were available to the Committee on these materials. An evaluation could not be made and no monograph was prepared.

Scarlet GN

The Committee was not aware of a source or use of Scarlet GN as a food colour. The specifications were not revised.

Studies available to the Committee consisted of limited mutagenic and short-term toxicity studies as well as a long-term study in the rat. The number of animals studied was insufficient and there was a lack of detail regarding the factors measured. No evaluation could be made and no monograph was prepared.

Silver

In view of the rare use of this metal and in the absence of knowledge of the exact nature of silver used on or in foods, specifications were not prepared. The data available suggest that this substance might accumulate in certain tissues following long-term exposure. There were, however, insufficient data to evaluate this point fully, nor were any adequate long-term studies available. Thus no evaluation could be made. A monograph was prepared.

Ultramarines

The Committee was not aware of a current use of ultramarines as food colours. The specifications were not revised. The available toxicity data, consisting of several short-term rat studies and a teratology study, could not be related to the specifications for these substances; hence no evaluation could be made. No monograph was prepared.

Yellow 2G

Tentative specifications were prepared for this substance. The results of long-term studies in mice and rats were available. A histopathological finding was a non-significant occurrence of lymphoma in mice treated with this substance. In the rat a treatment-related decrease in renal concentrating ability was noted. The no-effect level for this response was 100 mg/kg. On the basis of this study a temporary ADI for man of 0-0.025 mg/kg of body weight was established. Further work required comprises reproduction/teratology studies and studies of metabolism in several species, preferably including man. A monograph was prepared.

Xanthophylls

Earlier specifications for xanthophylls were reviewed. Xanthophylls are considered as belonging to the class of carotenoids (see the general discussion of food colours in section 2.2). Products available have so far been used chiefly as animal

(poultry) feed additives to enhance the colour of egg yolks and chicken fat. Two dry types are available: (1) citranaxanthin synthesized from beta-apo-8'-carotenal in reaction with acetone and (2) dried tagetes meal, ground flower petals of Aztec marigold (*Tagetes erecta* L.), or dried algae (genus *Spongiococcum*). Tentative specifications were prepared for citranaxanthin. Not enough information was available to enable the Committee to establish specifications for the other type of powdered product. No toxicity data were available on these materials; thus no evaluation could be made nor could an ADI be established. No monograph was prepared.

3.4 Sweeteners

Aspartame

Specifications were available to the Committee. The Committee noted that aspartame had been considered at two previous meetings (nineteenth and twentieth). At the nineteenth meeting a question had been raised of the possible significance of "uterine polyps" to the administration of the impurity diketopiperazine. The problem was considered to be of no significance by subsequent reviews carried out by three independent groups of pathologists.

The Committee had therefore concluded that the safety of aspartame had been adequately demonstrated and was prepared to establish an ADI. However, the task of the Committee had now been complicated by an assertion that the data base from which these conclusions were drawn requires validation.

The Committee therefore deferred its decision pending an assurance that the toxicological data are valid. No monograph was prepared.

Cyclamate, calcium and sodium salts

Specifications were available to the Committee.

The Committee noted that an unresolved problem in the toxicological evaluation of cyclamate concerns the metabolite cyclohexylamine (CHA) produced by gut flora. CHA is known to produce testicular atrophy in rats. This was considered to be a species-specific effect and it was not known whether it had ever occurred in human males. Several studies on the effects of CHA on the testes were considered by the Committee and it was concluded that the no-effect level for this response was about 75 mg/kg of body weight per day.

The Committee was also aware of a multigeneration mouse reproduction study. In this study there was a slight decrease in litter size and weight of newborn mice from parents fed 0.5% (500 mg/kg of body weight) cyclohexylamine hydrochloride. This effect was observed from the first generation onwards. It was noted by the Committee that the maternal weight loss and reduced food consumption observed

in CHA-treated animals may have contributed to the embryotoxic effects. A no-effect level for CHA embryotoxicity could not be determined and because of this the Committee recommended a temporary ADI until a no-effect level could be established.

Data were available indicating that about 40% of dietary cyclamate was absorbed and thus unavailable for conversion to cyclohexylamine. Conversion of cyclamate to cyclohexylamine has been assessed in humans. These studies indicated great variation between individuals, and it was noted that a given individual may convert up to 80% of cyclamate on one occasion but at another time may convert only a small amount. This variability was considered to be related to changes in the gut flora. It was estimated that for safety evaluation purposes an average figure of 30% conversion of the unabsorbed cyclamate could be accepted. Thus taking into consideration all these factors a temporary ADI was calculated as follows:

No-effect level of cyclohexylamine (free base) in rat = 74 mg/kg of body weight per day. Temporary ADI (200-fold safety factor) for man for cyclohexylamine = 0.37 mg/kg of body weight.

To convert an ADI for cyclohexylamine into an ADI for cyclamic acid it is necessary to multiply by the ratio of the molecular weight of cyclamic acid to the molecular weight of cyclohexylamine, which is equal to 2. Thus, if all cyclamate was converted to cyclohexylamine, the ADI for cyclamate would be 0.74 mg/kg of body weight.

Since only 60% of ingested cyclamate is available for conversion and only 30% of cyclamate is converted to cyclohexylamine, the temporary ADI for cyclamate is: $0.74 / (0.6 \times 0.3) \sim 4$ mg/kg of body weight, expressed as cyclamic acid.

On the basis of this calculation, a temporary ADI for man of 0-4 mg/kg of body weight was established.

The following studies are required by 1980:

- (1) studies to determine the no-effect level for CHA-induced embryotoxicity in the mouse.
- (2) studies to determine the effect of dose on the degree of cyclamate absorption prior to conversion to CHA.
- (3) studies to identify more precisely the no-effect level for CHA effects on the testes of the rat.
- (4) human studies to identify more precisely the percentage of conversion of cyclamate to CHA in the gut.

A condensed monograph was prepared.

Saccharin, potassium and sodium salts

Specifications were available.

Saccharin was evaluated by the Joint Expert Committee during the tenth and eighteenth meetings.¹ At that time an unconditional ADI for man of 5 mg/kg of body weight and a conditional ADI of 15 mg/kg of body weight were established.

In three carcinogenicity studies there was a significant incidence of bladder tumours in the F₁ generation of male rats fed at dietary levels of 5% saccharin or greater. In these studies, unlike previous long-term studies, animals were exposed *in utero* and through mothers' milk and consequently for their lifetime. There are few other compounds that have been tested employing animals exposed *in utero* in two-generation studies, and the desirable background information on this test procedure is not available.

The possibility that *o*-toluenesulfonamide (OTS), a major impurity in saccharin used in two of the above-mentioned studies, might be the causative agent has been ruled out because this substance does not in itself cause bladder tumours in the rat and because, when OTS-free saccharin was used, bladder tumours were still produced.

The overall findings remain unexplained. Saccharin has not produced bladder tumours in many conventional long-term feeding studies in several species. Studies on the mutagenicity of saccharin have produced both positive and negative results. The inconsistency of these results may be due to the presence of impurities in some of the samples tested. Saccharin *per se* does not conform to the characteristics of most other chemical carcinogens in that it is not known to be metabolized prior to excretion.

It is possible that an unidentified impurity may be responsible for these results, but if so it would be a potent carcinogen. It is also possible that saccharin is acting as a "promoting agent" to a carcinogen common to all three positive studies or that, at the high levels of saccharin used in the present experiments, its action is due to physical influences.

Epidemiological studies, primarily in diabetic populations have indicated no increased risk of bladder cancer. However, these studies suffer from disabilities in sample size, discrete sample populations, and inherent statistical limitations.

Owing to concern resulting from the new findings, which need further elucidation, the Committee changed the previous unconditional ADI for man of 0–5 mg/kg of body weight to a temporary ADI of 0–2.5 mg/kg and abolished the conditional ADI of 0–15 mg/kg of body weight for dietetic purposes only. The Committee was aware of studies in progress and therefore recommended that this substance be kept under review.

Further work required by 1980 includes the following investigations.

(1) Studies to determine if saccharin *per se* produces physiological changes that predispose to or account for the production of bladder tumours.

¹ See Annex 1, references 12 and 34.

(2) Chemical studies and short-term *in vitro* studies that would isolate and identify potential carcinogenic fractions in saccharin.

(3) Carcinogenicity tests to establish the oncogenic potential of active impurities.

(4) Studies to determine whether the action of active principles requires exposure *in utero* or exposure through suckling, or a combination of both these exposures together with long-term feeding exposure. These studies should include the genesis of the pathological lesion during embryonic development of bladder tissue and an examination of the transplacental pharmacokinetics of saccharin and its impurities.

(5) Studies to determine whether saccharin promotes the action of impurities or carcinogenic fractions normally found in animal diets.

(6) The initiation of prospective epidemiology in high-risk populations.

A condensed monograph was prepared.

Xylitol

Tentative specifications were prepared for this substance, on which a considerable amount of information was available to the Committee. Metabolic studies indicated that about 20% of dietary xylitol is absorbed. Xylitol enters the pentose monophosphate pathway of glucose in both animal and man, but has only a slight effect on insulin secretion. Long-term studies in rats and mice have not been completed and hence potential carcinogenicity cannot be assessed. In a two-year dog study, 10% of the diet appeared to be a no-effect level. In monkeys, softening of stools was noted at doses of 1–5 g per kg diet per day. When 52 human volunteers were fed 50–70 g/day of xylitol for two years, occasional slight diarrhoea was noted. No cataracts or instances of hepatic toxicity were noted in any of these studies.

The Committee was aware of some deaths caused in patients to whom xylitol had been administered intravenously for purposes of parenteral alimentation. The Committee considered that this effect probably had no relevance to food additive use. However, in the light of conflicting data on man, it was concluded that no evaluation of this substance should be made without a complete toxicological profile. No ADI was established. A monograph was prepared.

3.5 Thickening agents

Karaya gum

The existing specifications were revised, but further information is required on viscosity range. Specifications remain tentative. New data available on this com-

pound consisted of limited clinical observations in man and some negative mutagenicity data. The Committee considered these data to be inadequate for evaluating the toxicity of the material. No new monograph was prepared.

Oat gum

The Committee was not aware of a commercial source of oat gum as a food additive product. No information was available on the nature or uses of the oat gum produced for other purposes. Specifications were not prepared. No toxicological data were available on this substance, and hence no evaluation could be made. No monograph was prepared.

Tragacanth gum

The existing specifications were revised, but they remain tentative. Additional toxicological data were provided in teratology and mutagenicity studies, which gave negative results. It was brought to the attention of the Committee that tragacanth gum affects hepatic metabolism in the rat.¹ The data were not sufficient to evaluate this substance and no ADI was set. No new monograph was prepared.

3.6 Miscellaneous

Benzoin gum

This exudate of trees grown in South-East Asia occurs in two forms, which differ in their main flavouring constituents, one containing benzoate-type and the other cinnamate-type compounds. The tentative specifications drawn up by the Committee cover both types. No toxicological data were available on these materials. Thus no ADI could be established and no monograph was prepared.

Diatomaceous earth

Tentative specifications were prepared for diatomaceous earth as a filtration aid. This material includes an ill-defined group of silicious substances. The Committee was unable to relate the available toxicological studies to a grade of diatomaceous earth that would meet food additive specifications. Thus, no evaluation could be made. No monograph was prepared.

Hydroxylated lecithins

Specifications were available to the Committee. No new toxicological data were available on these substances and no ADI could be established. No new monograph was prepared.

¹ BACHMANN, E. & ZBINDEN, G. Biochemical effects of chemically administered suspending agents on mitochondrial metabolism and mixed function oxidases in the rat. *19th Meeting of the European Society of Toxicology, Copenhagen, June 19-22, 1977.*

Licorice

The Committee considered that the term "licorice" should not be used to describe a food colour. Products derived from licorice are used as food flavours. Specifications were not available for licorice. No toxicological data were available. No evaluation was made and no monograph was prepared.

Sucrose acetate isobutyrate

Tentative specifications were prepared for this substance, which was evaluated during the nineteenth meeting. At that time it was concluded that a two-year study on the dog should be carried out in order to assess the adverse effects of the substance on liver function. The results of this study are not yet available, and limited new metabolic studies are insufficient to resolve the question. No ADI was allocated. The Committee, in reviewing all available data in the light of the proposed use of this compound, concluded that a complete toxicological profile was needed.

Studies required to evaluate this compound are a carcinogenicity/toxicity study in two animal species, a two-year dog study with adequate numbers and dose groups to demonstrate a no-effect level, and a multigeneration reproduction/teratology study. No new monograph was prepared.

4. REVIEW OF SPECIFICATIONS

Activated vegetable carbon

The revised tentative specifications prepared apply to the substance when used as a processing aid, chiefly for its adsorptive capacity.

Azorubine (Carmoisine)

A method for determining free aromatic amines was added to the existing specifications, which remain tentative.

Brilliant black PN

A method for determining free aromatic amines was added to the existing specifications, which remain tentative.

"Chlorophyll" compounds

Although commercial practice and nomenclature of some products make use of the word "chlorophyll", the Committee recognized that changes in the native chlorophyll invariably occur during and after extraction of the colour principles from plant materials. Products having the chemical names and structural formulas given in the existing specifications are not available, nor do names and formulas

represent the colouring principles of food colour products. The existing specifications for "chlorophyll" are therefore revoked.

The Committee was aware that a number of food colours currently produced are based on extraction of the chlorophylls of plants. The nomenclature of these products is not widely agreed at present. A major differentiation derives from the use of copper salts (up to 1000 mg Cu/kg) to stabilize the food colour product. Copper salts may further be used at higher levels (above 1000 mg Cu/kg) to effect partial replacement of the magnesium (originally present in the porphyrin-type complex molecule of chlorophyll and carried forward into the products of degradation or change). Information is required concerning which food colours are described by the existing specifications for "chlorophyll copper complex" and particularly by a limit of 200 mg of free ionizable copper per kilogram. These specifications therefore remain tentative. The Committee suggests that a nomenclature should be agreed and that specifications should then be prepared for all acceptable food colours based on chlorophyll.

Substantial agreement exists on "chlorophyllin copper complex, sodium and potassium salts", and the existing specifications were revised by adding the required method for determining free ionizable copper.

Cyclic phosphates

This item was on the agenda primarily with regard to consideration of a limit for cyclic phosphates in pentasodium triphosphate and sodium polyphosphate, glassy (see below). However, it was noted that one particular cyclic phosphate, namely sodium trimetaphosphate, is used as a modifying agent for chemically treated starches. Consideration might therefore be given at a future meeting to preparing specifications for this substance.

Food Green S

A method for determining free aromatic amines was added to the existing specifications, which remain tentative.

Lactic acid

A limit test for cyanide ion was provided to complete the specifications prepared at the previous meeting of the Committee.

Magnesium silicate

The specifications were reviewed. The further information requested, i.e., the means to distinguish between the food and medicinal grades, had not become available. The specifications remain tentative.

Mineral oil

The specifications were revised. The specifications exclude mineral oils produced by the hydrogenation process unless subsequently refined by the use of oleum.

Patent Blue V

A method for determining free aromatic amines was added to complete the existing specifications, which remain tentative.

Pentasodium triphosphate and sodium polyphosphate, glassy

Specifications for pentasodium triphosphate and for sodium polyphosphate, glassy, were reviewed with regard to the content of cyclic phosphate. A separate provision for cyclic phosphates does not appear to be needed for pentasodium triphosphate because the assay requirement and method limit the presence of this form. More information, however, is needed on cyclic phosphates in sodium polyphosphate, glassy.

Ponceau 4R

The existing specifications were revised to provide for an assay of total dye, a limit for loss on drying, a limit on the content of chlorides and sulfates, and a method for determining free aromatic amines. The specifications remain tentative.

Quinoline Yellow

In addition to variations in the composition of a methyl substituent noted at a previous meeting, products manufactured in the USA are also predominantly in the disulfonated form whereas those produced in Europe are mainly monosulfonated. The Committee recommended that the existing tentative specifications be reviewed and updated when activities in progress to define the articles of commerce have brought results.

Titanium dioxide

The existing specifications were revised to take into account products manufactured using aluminium oxide or silicon dioxide to increase dispersibility.

Alpha-tocopherol and mixed tocopherols concentrate

The existing specifications were revised. For mixed tocopherols concentrate, the colorimetric assay method was replaced with a gas chromatographic determination of the individual isomers, preceded by column chromatographic treatment of the sample to eliminate interference by sterols.

Xanthan gum

The existing specifications were revised with respect to several of the methods given. Further information is required on residual nitrogen levels.

5. FUTURE WORK

(1) A number of food additives have been allocated temporary ADIs. These should be re-evaluated when the required information becomes available.

(2) Enzymes are finding increasing use in food manufacture as processing aids. They should therefore be included in a broader evaluation of processing aids by a future Committee at an early date.

(3) In discussing colour additives, the Committee recognized that colours may also be added to animal feed and be deposited as such or as metabolites in edible animal products, e.g., egg yolk and chicken skin. It is clear that such colours must be considered as food additives requiring toxicological evaluation. The problems related to specifications and safety evaluation of such colours should be discussed at a future meeting.

(4) Adequate background information should be assembled and made available to the Expert Committee in order to arrive at proposals for tolerable weekly intakes for infants and young children for lead, cadmium, mercury, and PCBs. The need to establish tolerable intakes for infants and young children for other toxic trace elements and organo-halogen compounds found as contaminants in food should be examined.

6. RECOMMENDATIONS TO FAO AND WHO

(1) In view of the rapid progress of the science of toxicology and the increasing refinement of evaluation procedures, the Committee felt strongly that the traditional concepts of setting ADIs, the application of safety factors, and the relationship of these safety factors to the observed toxicological manifestations in animal experiments should be reconsidered. It therefore proposed that these complex problems be a topic of future discussion.

(2) In the opinion of the Committee the monographs provide an important source of information for both developing and industrialized nations. It wishes to stress its support for continuing publication of these monographs by FAO and WHO.

(3) In view of the increasing amount of toxicological information and the large number of food additives, flavours, processing aids, packaging materials and con-

taminants to be evaluated or re-evaluated, it was considered that annual meetings of the Expert Committee on Food Additives would not be able to keep abreast of this task. FAO and WHO should consider ways of overcoming this problem.

(4) There is a need to develop new or improved guidelines for evaluating the long-term effects of food additives and contaminants on the fetus exposed *in utero* and on the neonate exposed via the mother's milk. In addition, guidelines should be developed to assure the safety of food additives in the diet of infants not being nursed. These subjects should form topics for future discussion.

(5) In view of the special susceptibility of the fetus, infants, and young children to the harmful effects of many food contaminants, countries collaborating in the FAO/WHO food and animal feed contamination monitoring programme should pay close attention to the foods eaten by infants and young children particularly with regard to contaminants such as PCBs, aflatoxins, lead, cadmium, and mercury. Surveys should be made in developing countries, taking into account the special diets existing in these countries.

(6) It is recognized that microbiological criteria are needed for certain food additives (see section 2.5), and that a consistent rationale is required in their application and inclusion in specifications. This need should be met as soon as possible, either through the use of consultants to prepare documentation for consideration by an early future meeting of the Committee or through a separate meeting of experts.

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

Documents marked with an asterisk may be obtained on request from : Food Additives, World Health Organization, 1211 Geneva 27, Switzerland, or from Food Standards and Food Science Service, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy.

1. *General principles governing the use of food additives*. (First report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 15, 1957 ; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use*. (Second report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 17, 1958 ; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)*. (Third report of the Expert Committee). 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 (out of print).
4. *Specifications for identity and purity of food additives (food colours)*. (Fourth report of the Expert Committee). 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 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives*. (Fifth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 29, 1961 ; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants*. (Sixth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 31, 1962 ; WHO Technical Report Series, No. 228, 1962.
7. *Specifications for the identity and purity of food additives and their toxicological evaluation : emulsifiers, stabilizers, bleaching and maturing agents*. (Seventh report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 35, 1964 ; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation : food colours and some antimicrobials and antioxidants*. (Eighth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 38, 1965 ; WHO Technical Report Series, No. 309, 1965 (out of print).

- *9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants.* FAO Nutrition Meetings Report Series, No. 38A, 1965 ; WHO/Food Add/24.65.
- *10. *Specifications for identity and purity and toxicological evaluation of food colours.* FAO Nutrition Meetings Report Series, No. 38B, 1966 ; 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 of the Expert Committee). FAO Nutrition Meetings Report Series, No. 40, 1966 ; WHO Technical Report Series, No. 339, 1966.
12. *Specifications for the identity and purity of food additives and their toxicological evaluation : some emulsifiers and stabilizers and certain other substances.* (Tenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 43, 1967 ; WHO Technical Report Series, No. 373, 1967.
- *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 of the Expert Committee). FAO Nutrition Meetings Report Series, No. 44, 1968 ; WHO Technical Report Series, No. 383, 1968.
- *15. *Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968 ; 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, No. 44B, 1969 ; WHO/Food Add/69.31.
17. *Specifications for the identity and purity of food additives and their toxicological evaluation : some antibiotics.* (Twelfth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 45, 1969 ; WHO Technical Report Series, No. 430, 1969.
- *18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Report Series, No. 43A, 1969 ; 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 of the Expert Committee). FAO Nutrition Meetings Report Series, No. 46, 1970 ; WHO Technical Report Series, No. 445, 1970.
- *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 of the Expert Committee). FAO Nutrition Meetings Report Series, No. 48, 1971 ; WHO Technical Report Series, No. 462, 1971.
- *23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971 ; 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, No. 48B, 1971 ; WHO/Food Add/70.40.
- *25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971 ; 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 of the Expert Committee). FAO Nutrition Meetings Report Series, No. 50, 1972 ; WHO Technical Report Series, No. 488, 1972.
27. *Toxicological evaluation of some enzymes, modified starches and certain other substances.* FAO Nutrition Meetings Report Series, No. 50A, 1972 ; WHO Food Additives Series, No. 1, 1972.
28. *Specifications for the identity and purity of some enzymes and certain other substances.* FAO Nutrition Meetings Report Series, No. 50B, 1972 ; WHO Food Additives Series, No. 2, 1972.
29. *A review of the technological efficacy of some antioxidants and synergists.* FAO Nutrition Meetings Report Series, No. 50C, 1972 ; WHO Food Additives Series, No. 3, 1972.
30. *Evaluation of certain food additives and the contaminants mercury, lead and cadmium.* (Sixteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 51, 1972 ; WHO Technical Report Series, No. 505, 1972, and corrigendum.
31. *Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbonate and octyl gallate.* FAO Nutrition Meetings Report Series, No. 51A, 1972 ; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications.* (Seventeenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 53, 1974 ; WHO Technical Report Series, No. 539, 1974, and corrigendum.
33. *Toxicological evaluation of certain food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A ; WHO Food Additives Series, No. 5, 1974.
34. *Evaluation of certain food additives.* (Eighteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 54, 1974 ; WHO Technical Report Series, No. 557, 1974, and corrigendum.
35. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 54A, 1975 ; WHO Food Additives Series, No. 6, 1975.

36. *Specifications for the identity and purity of some food colours, flavour enhancers, thickening agents and certain food additives.* FAO Nutrition Meetings Report Series, No. 54B, 1975 ; WHO Food Additives Series, No. 7, 1975.
37. *Evaluation of certain food additives : some food colours, thickening agents, smoke condensates, and certain other substances.* (Nineteenth report of the Expert Committee). FAO Nutrition Meetings Report Series, No. 55, 1975 ; WHO Technical Report Series, No. 576, 1975.
38. *Toxicological evaluation of some food colours, thickening agents, and certain other substances.* FAO Nutrition Meetings Report Series No. 55A. WHO Food Additives Series, No. 8, 1975.
39. *Specifications for the identity and purity of certain food additives.* FAO Nutrition Meetings Report Series, No. 55B, 1976 ; WHO Food Additives Series, No. 9, 1976.
40. *Evaluation of certain food additives.* (Twentieth report of the Expert Committee). FAO Food and Nutrition Series, No. 1, 1976 ; WHO Technical Report Series, No. 599, 1976.
41. *Toxicological evaluation of certain food additives.* FAO Food and Nutrition Series, No. 1A, 1978 ; WHO Food Additives Series, No. 10, 1978.
42. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Series, No. 1B, 1977 ; WHO Food Additives Series, No. 11, 1977.

Annex 2

ACCEPTABLE DAILY INTAKES AND INFORMATION ON SPECIFICATIONS

	Specifications ¹	ADI for man (mg/kg body weight)
<i>Acids, bases and salts</i>		
adipic acid	S	0-5
monosodium L-(+)-tartrate	S	0-30 ²
DL-tartaric acid (synthetic)	NT	No ADI allocated
<i>Antioxidants</i>		
tertiary butyl hydroquinone (TBHQ)	RT	0-0.5 ³
<i>Food colours</i>		
alkanet and alkanin	O ⁴	No ADI allocated
Allura Red AC	NT	Decision postponed
aluminium	N	No ADI allocated ⁵
anthocyanins	S	No ADI allocated
anthocyanin colour from grapeskins	NT	No ADI allocated
Benzyl Violet 4B	S	No ADI allocated ⁶
Black 7984	S	No ADI allocated
Brown FK	S	No ADI allocated
capsanthine	O	Decision postponed
capsorubine	O	Decision postponed
caramel colours (ammonia process)	RT	No ADI allocated ⁷
caramel colours (ammonia-sulfite process)	NT	0-100 ⁸
carbon black	S	Decision postponed
carthamus (yellow)	NT	No ADI allocated
chlorophyllin copper complex (sodium and potassium salts)	R	0-15 ⁹
Chocolate Brown FB	O	Decision postponed
Chocolate Brown HT	RT	0-0.25 ⁸
Chrysoine	S	Decision postponed
cochineal, carmine and carminic acid	S	No ADI allocated
carmine: aluminium and aluminium calcium lakes of carminic acid	NT	No ADI allocated
Fast Red E	RT	Decision postponed
Fast Yellow AB	ST	Decision postponed
gold	O	No ADI allocated ⁵
Lithol Rubine BK (calcium salt)	NT	Decision postponed
lycopene	O	Decision postponed
Orange I	ST	No ADI allocated
Orange GGN	ST	Decision postponed
Orange RN	ST	No ADI allocated
Persian Berries	O	Decision postponed
Ponceau 6R	S	Decision postponed
Ponceau SX	S	No ADI allocated

	<i>Specifications¹</i>	<i>ADI for man (mg/kg body weight)</i>
Quercitin and quercitron	S	No ADI allocated
Red 2G	RT	0–0.006 ^a
saffron, crocin and crocetin	RT (saffron)	Decision postponed
Scarlet GN	S	Decision postponed
silver	O	Decision postponed
ultramarines	S	Decision postponed
Yellow 2G	NT	0–0.025 ^a
xanthophylls	S	No ADI allocated
<i>Sweetening agents</i>		
aspartame	ST	Decision postponed
cyclamate, calcium and sodium salts	S	0–4 ^a
saccharin, potassium and sodium salts	S	0–2.5 ^a
xylitol	NT	No ADI allocated
<i>Thickening agents</i>		
karaya gum	RT	Decision postponed
oat gum	O	Decision postponed
tragacanth gum	RT	No ADI allocated
<i>Miscellaneous food additives</i>		
benzoin gum	NT	No ADI allocated
diatomaceous earth	NT	Decision postponed
hydroxylated lecithins	S	No ADI allocated
licorice (flavour)	O	Decision postponed
sucrose acetate isobutyrate	NT	No ADI allocated

Specifications only

<i>Substance</i>	<i>Specification¹</i>
activated vegetable carbon	R
Azorubine (Carmoisine)	RT
Brilliant Black PN	R
caramel colours (plain)	RT
carthamus (red)	NT
citranaxanthin	N
Food Green S	RT
lactic acid	R
magnesium silicate	ST
mineral oil	R
mixed tocopherols concentrate	R
Patent Blue V	RT
Ponceau 4R	RT
Quinoline Yellow	RT
titanium dioxide	R
alpha-tocopherol	R
xanthan gum	RT

Notes to tables in Annex 2.

¹ N, new specifications prepared; O, specifications not prepared; R, existing specifications revised; S, specifications exist, revision not considered; T, the existing, new, or revised specifications are tentative and comments are invited.

² Expressed as L-(+)-tartaric acid.

³ As BHA, BHT, TBHQ, or the sum of the three compounds.

⁴ Existing specifications were revoked.

⁵ The use is very limited. Not considered to present a hazard.

⁶ Not to be used in food.

⁷ Previous temporary ADI revoked.

⁸ Temporary acceptance.

⁹ The previous temporary ADI was converted to an ADI.

Annex 3

FURTHER TOXICOLOGICAL STUDIES AND INFORMATION REQUIRED

*Allura Red AC*¹

See WHO Food Additives Series, No. 6, 1975, p. 41.

*Aspartame*¹

Assurance that the toxicological data are valid.

*Caramel colours (ammonia-sulfite process)*³

Adequate carcinogenicity/teratogenicity studies with particular attention to bone marrow and immune competence. Attempts should be made to identify the principle responsible for the observed effects.

*Chocolate Brown HT*²

- (1) Multigeneration reproduction/teratology studies.
- (2) Metabolic studies in several animal species, preferably including man.

*Cyclamate, calcium and sodium salts*³

- (1) Studies to determine the no-effect level for cyclohexylamine-induced embryotoxicity in the mouse.
- (2) Studies to determine the effect of dose on the degree of cyclamate absorption prior to conversion to CHA.
- (3) Studies to identify more precisely the no-effect level for CHA effects on the testes of the rat.
- (4) Human studies to identify more precisely the percentage of conversion of cyclamate to CHA in the gut.

*Ponceau SX*¹

- (1) Studies in the dog to define a no-effect level for the effects observed in previous studies, namely the atrophy of the adrenal zona glomerulosa, haemorrhagic lesions, and blood-filled mucosal projections into the urinary bladder.
- (2) A multigeneration reproduction/teratology study.

*Red 2G*²

- (1) A multigeneration reproduction/teratology study.
- (2) Studies on the bone marrow to elucidate the toxic effects on erythropoiesis.

*Saccharin, potassium and sodium salts*³

- (1) Studies to determine if saccharin *per se* produces physiological changes that predispose to or account for the production of bladder tumours.
- (2) Chemical studies and short-term *in vitro* studies that would isolate and identify potential carcinogenic fractions in saccharin.
- (3) Carcinogenic tests to establish the oncogenic potential of active impurities.
- (4) Studies to determine whether the action of active principles requires exposure *in utero* or exposure through suckling, or a combination of both these exposures together with long-term feeding

exposure. These studies should include the genesis of the pathological lesion during embryonic development of bladder tissue and an examination of the transplacental pharmacokinetics of saccharin and its impurities.

(5) Studies to determine whether saccharin promotes the action of impurities or carcinogenic fractions normally found in animal diets.

(6) The initiation of prospective epidemiology in high-risk populations.

*Sucrose acetate isobutyrate*¹

(1) A carcinogenicity/toxicity study in two animal species.

(2) A two-year dog study with adequate number of animals and dose groups to demonstrate a no-effect level.

(3) A multigeneration reproduction/teratology study.

*Yellow 2G*²

(1) Reproduction/teratology studies.

(2) Metabolic studies in several animal species, preferably including man.

¹ Further work required before an acceptable daily intake for man can be allocated.

² Information required by 1979.

³ Information required by 1980.