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EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Thirty-seventh report of the
Joint FAO/WHO Expert Committee on
Food Additives

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CORRIGENDA

Page 17, lines 31 and 33–34:

Delete ...10 mg/kg in the diet...
Insert ...10 g/kg in the diet...

Page 18, lines 11–12:

Delete ...2.5 mg/kg in the diet...
Insert ...2.5 g/kg in the diet...

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Joint FAO/WHO Expert Committee on Food Additives

Geneva, 5–14 June 1990

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 28, in press.

Specifications are issued separately by FAO under the title:

Specifications for the identity and purity of certain food additives. (To be published as an FAO Food and Nutrition Paper.)

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 5 to 14 June 1990. The meeting was opened by Dr W. Kreisel, Director, Division of Environmental Health, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr Kreisel noted that the recommendations made at previous meetings of the Committee had been used by Member States of FAO and WHO to formulate national regulations on food additives and by the Codex Alimentarius Commission. The Committee's recommendations had probably contributed more to the elaboration of sound national food legislation than had those of any other international body concerned with problems of food technology and safety.

Dr Kreisel drew attention to the forthcoming Joint FAO/WHO Conference on Food Standards, Chemicals in Food, and Food Trade which was scheduled to be held in Rome, in March 1991. This conference would consider certain issues concerning the Codex Alimentarius and the food trade in addition to food additives and contaminants and residues of pesticides and veterinary drugs in food. It would be the first such conference in 20 years and would point the way for future activities of the Committee.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955,[1] there have been 36 previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the recommendation made at the thirty-fifth meeting (Annex 1, reference *88*).

The tasks before the Committee were: (a) to undertake toxicological evaluations of certain food additives and contaminants; (b) to review and prepare new or revised specifications for selected food additives; (c) to discuss and advise on matters arising from the eighteenth session of the Codex Alimentarius Commission and the twenty-second session of the Codex Committee on Food Additives and Contaminants (Annex 4); and (d) to discuss the effectiveness and safety of the long-term use of potassium iodate and potassium iodide for fortifying salt, as requested by the Forty-third World Health Assembly (Annex 5).

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[1] *Joint FAO/WHO Conference on Food Additives.* FAO Nutrition Meetings Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956.

2.1 Modification of the agenda

Acesulfame potassium was added to the agenda for toxicological evaluation. In addition, the Committee responded to a request made in May 1990 by the Forty-third World Health Assembly, as noted above.

The original list of substances to be evaluated at this meeting included "chymosins A and B from bacterial sources". However, data were received not only on chymosin from a bacterial source (*Escherichia coli* K-12), but also on preparations from a fungus (*Aspergillus niger* var. *awamori*) and from a yeast (*Kluyveromyces lactis*). All three types of chymosin were therefore reviewed by the Committee.

Also included in the original list of substances for evaluation was "α-amylase from *Bacillus subtilis*". Since data were, in addition, received on an α-amylase from *B. stearothermophilus* expressed in *B. subtilis*, both α-amylases were reviewed by the Committee.

The substance "isoascorbic acid" in the original list of substances was evaluated by the Committee under the name "erythorbic acid".

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*). This publication, developed in response to repeated recommendations by the Committee, embraces the major observations, comments, and recommendations on the safety assessment of food additives and contaminants contained in the previous reports of the Committee and other associated bodies. The Committee noted that the document reaffirms the validity of recommendations that are still appropriate, and points out the problems associated with those that are no longer valid in the light of modern technical advances.

2.2.1 *The role of pharmacokinetics in the safety evaluation of food additives and contaminants*

The Committee drew attention to the previously published statement on "The use of metabolic and pharmacokinetic studies in safety assessment" in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), and to the role of such studies in facilitating the extrapolation of data from one species to another, particularly at high levels of exposure.

The safety assessment of a food additive or contaminant is, of necessity, largely based upon the results of studies in experimental animals. Only rarely are adequate human data available to aid this process. The extrapolation of animal toxicity data to humans is complicated by the

occurrence of interspecies differences in both the disposition process (absorption, distribution, metabolism and excretion) and the response mechanisms to chemical substances. This is particularly true of long-term toxicity studies in which high doses are used. In evaluating the safety of chemical substances for use in humans, priority should therefore be given to minimizing such problems, so as to facilitate decision-making. In this regard, the Committee takes account of developments in appropriate areas, such as pharmacokinetics, biochemistry, toxicology and cell and molecular biology, which may be helpful in improving the accuracy of interspecies extrapolation for the purposes of safety assessment.

The Committee stated that it wished to encourage and promote a more widespread use of pharmacokinetic studies in the process of safety evaluation. Attention was drawn to the fact that dosage, as expressed in the conventional manner (e.g., administered dose in mg per kg of body weight), is not necessarily equivalent to systemic exposure and the level producing a toxic effect at the target site. This fact becomes particularly relevant when studies are performed in different animal species and data are extrapolated to humans, because of species, gender, and strain differences in the absorption, tissue distribution, biotransformation and excretion processes. Furthermore, the use of very high dose levels (e.g., the maximum tolerated dose) in long-term toxicity studies may cause phenomena such as "metabolic switching" due to the saturation or exhaustion of the normal metabolic pathways or the induction of enzymes, which can alter the "normal" biotransformation processes. The extent of such metabolic deviations may vary from species to species, and can have a large effect on exposure to the compound of interest at the target site.

The Committee recommended that appropriate pharmacokinetic data from various species, including where possible humans, should accompany future submissions of data from short-term and long-term studies. The Committee pointed out the advantages of including the investigation of pharmacokinetic parameters in acute studies before commencing short-term and long-term studies, as the results obtained can be of value in study design. Additional animals might be required in short-term and long-term studies, to be used primarily for investigating toxicokinetic and metabolic parameters. Such studies should be designed in order to determine the levels of systemic exposure at different dose levels used and whether metabolic switching and enzyme induction occur. The availability of such data will facilitate interspecies assessment of systemic exposure to a given compound, which will result in the generation of a much more reliable "safety factor" than one based upon dosage considerations alone.

2.2.2 *Carcinogenic food contaminants*

The Committee was asked to review the effects of benzo[*a*]pyrene, a ubiquitous carcinogenic chemical. A major reason for the occurrence of benzo[*a*]pyrene in food is the use of certain types of heat processing, such

as smoking, baking and cooking. Heat processing can result in the production of numerous other carcinogenic polycyclic aromatic hydrocarbons, various nitrosamines, aminoimidazoazarenes (in cooked meats), and various heterocyclic amines formed as pyrolysis products of certain amino acids. Toxicologists are therefore faced with the evaluation of the potential hazard of a large range of chemicals that occur as contaminants in human food and are carcinogenic in animals. The Committee emphasized, however, that toxicological effects other than carcinogenicity are not necessarily less important in the safety evaluation of such compounds.

Benzo[*a*]pyrene invariably occurs in association with many other carcinogenic polycyclic aromatic hydrocarbons. Attention has been focused on benzo[*a*]pyrene mainly because it has been analysed more frequently and subjected to extensive toxicological investigation. Recently interest has been focused on other compounds produced during cooking of meat, in particular certain heterocyclic amines and aminoimidazo-azarenes.

Advances in analytical chemistry and ongoing programmes of testing are disclosing an increasing number of carcinogenic contaminants in foods. Potentially carcinogenic nitrosamines may be produced in foods treated with nitrites (e.g., bacon) or exposed to nitrogen oxides (e.g., barley, during roasting for beer production). Other examples are the carcinogenic contaminants aflatoxins and ochratoxin A.

Various suggestions have been made over several decades that certain gastrointestinal tumours in humans are associated with the consumption of smoked foods. All the epidemiological observations have, however, been considered equivocal by the scientific community. Some reassurance that the risks are low is provided by the observation that contaminants in smoked foods are usually present at very low levels, and are associated with food processes dating back many centuries.

The Committee considered that potentially carcinogenic compounds in food should be identified and data collected on their occurrence, the influence of food technology processes on their levels in food, and their carcinogenic properties, so as to set priorities for assessing their safety.

2.2.3 *Safety assessment of flavouring agents*

The Committee reviewed the safety assessment of flavouring agents with respect to the particular features of this group of food additives, namely, the number of substances concerned, their diversity, and the low levels of use of many flavouring agents. Factors that should be considered in the safety evaluation of these compounds include: data from toxicological studies in animals and short-term tests for mutagenicity and clastogenicity, results from studies on metabolism and structure–activity relationships, the level of usage, the consumption ratio (Annex 1, reference *83,* Annex 4),

the source of the flavouring agent and data on the extent and frequency of human exposure (Annex 1, reference *76*).

In assessing the safety of flavouring agents, the Committee also takes into account the findings of other groups and institutions involved in the evaluation of these agents.

At its present meeting, the Committee considered the safety of three allyl esters of fatty acids, which were placed on the agenda on the basis of application of the method used in setting priorities for the safety review of food flavouring ingredients (Annex 1, reference *83*, Annex 4). The Committee concluded that a group Acceptable Daily Intake (ADI) should be allocated to the three esters on the basis of the allyl alcohol moiety, because they are rapidly hydrolysed and the observed toxic effects are due to this moiety. At the same time, it was recognized that a large number of other allyl esters of fatty acids are rapidly hydrolysed in a similar manner, so the same considerations should apply to them. It would have been helpful if data on these other substances had also been collected, so that they could have been considered for inclusion in the group ADI.

The Committee concluded that the safety evaluation of a specific flavouring agent would be facilitated by consideration of the structurally related group as a whole. Therefore, in most cases, all members of a structurally related group should be placed on the agenda, even if some of them are not in the highest priority level for evaluation.

2.3 Principles governing the establishment and revision of specifications

2.3.1 *Revision of the "Guide to specifications"*

The "General methods" section of the *Guide to specifications* (Annex 1, reference *65*) has not been updated since 1983. The need to update the general methods, to take account of changes in specifications, methodologies, and analytical techniques adopted since the last revision, was stressed at the Committee's last three meetings at which food additives were evaluated (Annex 1, references *77, 83* and *88*).

At its present meeting, the Committee considered a draft for a revised *Guide to specifications* to be published by FAO. The proposed changes included incorporation into the revised "General methods" section of the general methods for the analysis of food colours, which were prepared at the Committee's twenty-eighth meeting and partially revised at the thirty-first meeting (Annex 1, references *66* and *77*), and of other methods whose development had been requested at past meetings, such as those for infrared spectroscopy, gas chromatography (using headspace sampling) and microbiological tests. The Committee agreed to the proposed changes and took them into account in preparing specifications at its present meeting.

The Committee noted that the "General notices" section of the *Guide to specifications* – which provides guidance and definitions to assist in the preparation of specification monographs – was also being revised to take into account the changes and current developments in specifications, and a separate guide for enzyme preparations was being prepared.

2.3.2 *Microbiological criteria*

In revising the specifications of xanthan gum and establishing specifications for gellan gum, both of which are derived from microbiological sources, the Committee considered the microbiological criteria that should be specified. The Committee concluded that it was sufficient to specify limits and tests for total plate count, yeasts and moulds, coliforms, and salmonellae for these gums. These microbiological criteria are used widely for natural substances and are considered sufficient for the purpose of quality assurance. Additional requirements for the specific identity of the microorganisms in the final product were not considered necessary where the manufacturing process would essentially preclude such contamination.

2.4 Principles governing consideration of enzyme preparations from genetically modified microorganisms

The Committee has, on several previous occasions, addressed problems associated with the formulation of specifications for enzyme preparations used in food processing and manufacture. At its present meeting, the Committee had, for the first time, been asked to evaluate specific enzyme preparations from genetically manipulated source organisms. Consideration of the method of production and the specification of identity and purity is an important part of the safety evaluation of any food additive. The use of genetic modification techniques introduces factors additional to those associated with conventionally produced enzyme preparations.

Some assurance as to safety may be derived from the similarity of the active component of an enzyme preparation from transgenic sources to that from enzyme preparations produced conventionally and previously evaluated and considered to be safe. It is also important to ensure that harmful impurities have not been introduced into the final product by the organism serving as the source of the genetic material, during the process used in cloning the genetic material to be transferred or the construction of the production organism, or otherwise as a result of the genetic manipulation techniques employed. The final product should be characterized accordingly, and attention paid, for example, to the possible presence of viable cells of the source (transgenic) organism, the expression plasmid or vector, DNA fragments and non-enzyme protein.

The possibility of a latent capacity for toxin production by the donor or the host organism must also be taken into account. In this regard, the identity of the organisms serving as donors and intermediate and final hosts of the

transposed genetic material is crucial. A previous history of human exposure to, or investigation of, these organisms will be important in determining the extent of testing required, including toxicological testing of the end-product. Whenever such a product is to be evaluated by the Committee, a fully documented taxonomic history of the organisms concerned, together with detailed methods for their identification, should be provided. Furthermore, the use of national and international culture collections as sources of reference material by manufacturers to assist in the identification of microorganisms used commercially should be encouraged.

The Committee prepared an addendum to its previously published "General specifications for enzyme preparations used in food processing" (Annex 1, reference *69*) to reflect the additional concerns relating to enzyme preparations from genetically manipulated organisms. The Committee noted that the possibilities afforded by the techniques of biotechnology and genetic manipulation had implications not only for the development of new sources of enzymes but also for the production of other classes of food additives.

3. Comments on specific food additives and contaminants

The Committee evaluated a number of food additives and contaminants for the first time and re-evaluated several substances considered at previous meetings. Information on the evaluations and on specifications is summarized in Annex 2. Details of further toxicological studies and of other information required or desired for certain substances are given in Annex 3.

3.1 Specific food additives

3.1.1 *Antioxidants*

Butylated hydroxytoluene (BHT)

Butylated hydroxytoluene (BHT) was last evaluated at the thirtieth meeting of the Committee (Annex 1, reference *73*) when a temporary ADI of 0–0.125 mg per kg of body weight was established. At that time, the Committee based its evaluation of BHT on a one-generation reproduction study in rats, in which a no-effect level of 25 mg per kg of body weight per day was observed. As a long-term study in Wistar rats involving exposure to BHT *in utero* had shown hepatocarcinogenicity in male rats at a high dose level, in contrast to several previously reviewed single-generation long-term studies in Fischer 344 and Wistar rats, the Committee requested further investigation of the hepatocarcinogenicity of BHT in rats after *in utero* exposure. The Committee also noted that, in several studies from one laboratory, feeding of high doses of BHT caused haemorrhage in rats given

a diet containing low amounts of vitamin K, which suggested an anti-vitamin K effect of BHT. The Committee therefore requested further studies on the mechanism of the haemorrhagic effect of BHT.

The requirements of the Committee have been partially met. In further studies on the haemorrhagic effect of BHT in male Sprague-Dawley rats, the compound caused a very rapid decrease in levels of vitamin K-dependent coagulation factors in the plasma, while platelet aggregation did not seem to be affected initially. The causative agent is probably a metabolite of BHT, as it was demonstrated that inhibitors of hepatic drug metabolism reduced the effect on coagulation factors. The Committee noted that high doses of BHT are required to cause haemorrhage in vitamin K-deficient rats; it did not consider this effect to be critical with respect to the safety evaluation of BHT as a food additive in the human population.

The Committee was informed that a study had been initiated on the development and role of hepatic changes in long-term toxicity in male Wistar rats after exposure to BHT *in utero*. The Committee reviewed results from a "range-finding" study and from the main study in which the F_1 generation had been exposed to BHT in the diet for 7 months after weaning. The study design was very similar to that of the previously reported long-term study in which rats were exposed to the compound *in utero*.

Additional studies have confirmed that BHT is not genotoxic, and several short-term toxicity studies in rats have indicated that doses of up to 25 mg per kg of body weight per day have no toxic effects on the liver; high doses (250 mg per kg of body weight per day and above) are required to induce hepatic necrosis. In addition, the Committee noted that, in contrast to phenobarbital and DDT, BHT administered for 22 weeks did not increase the incidence of hepatocellular carcinomas in Wistar rats after initiation with dimethylnitrosamine.

The Committee extended the previously established temporary ADI of 0–0.125 mg per kg of body weight pending the results of the ongoing long-term study in rats involving *in utero* exposure to BHT. The Committee requested the final results of this study for re-evaluation of BHT in 1994.

An addendum to the toxicological monograph was prepared. The existing specifications for BHT were revised.

tert-Butylhydroquinone (TBHQ)

tert-Butylhydroquinone (TBHQ) was previously evaluated at the nineteenth, twenty-first, and thirtieth meetings of the Committee (Annex 1, references *38, 44,* and *73*). At the thirtieth meeting, a temporary ADI of 0–0.2 mg per kg of body weight was established based on the results of a long-term feeding study in dogs in which a no-effect level of 1.5 g/kg of the diet was observed. The Committee requested additional information for re-evaluation of the compound in 1990, including the results of lifetime

feeding studies in two rodent species. Additional studies to resolve questions related to the genotoxicity of TBHQ were considered desirable.

At its present meeting, the Committee reviewed data on the induction of hyperplasia, tumour-promoting activities, lung toxicity, and genotoxicity. The available *in vitro* and *in vivo* mutagenicity studies were equivocal, and did not rule out the possibility that the compound could be genotoxic. Although no adequate carcinogenicity study was available, the Committee was aware of ongoing long-term toxicity studies in rodents. The Committee therefore extended the temporary ADI of 0–0.2 mg per kg of body weight until 1994, when the results of these long-term studies should be made available for evaluation.

For the re-evaluation of TBHQ, additional data on genotoxicity, obtained by current techniques, would be desirable. Mechanistic studies to determine whether metabolites of TBHQ or reactive oxygen species produced by it are responsible for its possible genotoxic activity may help explain the equivocal results obtained so far.

A toxicological monograph was not prepared. The existing specifications for *tert*-butylhydroquinone were revised.

Erythorbic acid and its sodium salt

Erythorbic acid was previously evaluated at the sixth and seventeenth meetings of the Committee under the name isoascorbic acid (Annex 1, references *6* and *32*). The name was changed to erythorbic acid in accordance with the "Guidelines for designating titles for specifications monographs" adopted at the thirty-third meeting of the Committee (Annex 1, reference *83*). At the last evaluation an ADI of 0–5 mg per kg of body weight was allocated based on a long-term study in rats. At its present meeting, the Committee reviewed the results of new toxicological studies on erythorbic acid and its sodium salt, and studies on the metabolic and nutritional interactions of erythorbic acid with ascorbic acid.

In embryotoxicity and teratogenicity studies in rodents, erythorbic acid was without effect at dose levels up to 1 g per kg of body weight and the Committee did not consider that positive results obtained in chick embryo tests were indicative of potential teratogenicity or fetotoxicity in humans.

New long-term toxicity and carcinogenicity studies in rats and mice did not show any specific toxic or carcinogenic effects up to the maximum tolerated dose and most genotoxicity studies were negative. Studies on tumour promotion were also negative with the exception of those on bladder tumours initiated by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine in which high doses of sodium erythorbate (but not free erythorbic acid) showed effects. Similar effects were seen with sodium ascorbate (but not ascorbic acid) and various other sodium salts and the Committee concluded that this was not a specific effect of erythorbate.

Erythorbic acid is much more poorly absorbed and retained in the tissues than ascorbic acid; it is poorly reabsorbed in the kidney and rapidly

excreted. As a result it has low anti-scorbutic activity and only interferes significantly with ascorbic acid uptake and retention in the tissues when concentrations are at least an order of magnitude higher than those of ascorbic acid. Human studies showed that daily doses of 600 mg had no adverse effects on ascorbic acid repletion in volunteers depleted of vitamin C.

An ADI "not specified" was allocated to erythorbic acid and its sodium salt.

A toxicological monograph was prepared. The existing specifications for erythorbic acid were revised. The existing specifications for sodium erythorbate were not reviewed.

3.1.2 *Enzyme preparations*

α-Amylase from Bacillus stearothermophilus

α-Amylase produced extracellularly by the controlled fermentation of *Bacillus stearothermophilus* (ATCC 39709) was evaluated for the first time by the Committee at its present meeting.

This α-amylase preparation produced no significant toxicological effects in a 13-week feeding study in dogs up to a level of 0.28 g per kg of body weight per day nor in a one-generation (one-litter) reproduction study in rats in which some of the offspring were treated up to a level of 0.98 g per kg of body weight per day for 13 weeks after weaning.

An ADI "not specified" was allocated to this enzyme preparation.

A toxicological monograph and new specifications were prepared.

α-Amylase from Bacillus subtilis

α-Amylase produced extracellularly by the controlled fermentation of *Bacillus subtilis* strain F (ATCC 23350, DSM 7) was assessed for the first time by the Committee. Although only acute, 2-week, and 4-week studies in rats were available, in which levels of up to 100 mg/kg in the diet elicited no adverse effects, the Committee was able to draw upon the previous evaluation of mixed microbial carbohydrase and protease from *B. subtilis* (strain not identified) (Annex 1, reference *27*). In accordance with the earlier findings, the Committee allocated an ADI "not specified" to the α-amylase preparation.

A toxicological monograph and new specifications were prepared.

α-Amylase from Bacillus stearothermophilus expressed in Bacillus subtilis

α-Amylase produced extracellularly by the controlled fermentation of *Bacillus subtilis* (ATCC 39705) containing the gene for α-amylase from *B. stearothermophilus* (ATCC 39709) inserted by plasmid pCPC720 has not been previously evaluated by the Committee. At its present meeting, the

Committee reviewed the available data pertaining to the genetic modification procedures employed, characterization of the producing organisms, the fermentation process, and acute, short-term, and reproduction studies with the lyophilized enzyme preparation.

The Committee noted that a well-documented non-pathogenic and non-toxigenic strain of microorganisms had been employed in the genetic modification procedures. The vector used, pUB110, is well characterized and has been used for several years as a cloning vehicle for *B. subtilis*. The plasmid construct pCPC720, containing the α-amylase gene, was introduced into *B. subtilis* (ATCC 39705) using standard transformation procedures. The available data indicated the absence of antibiotic resistance, of production of "Shiga-like" toxin and of production of enterotoxins A, B, C and D by the α-amylase-producing microorganism.

The *B. subtilis* was grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation. The fermentation broth was filtered and the filtrate lyophilized before being mixed into the test diets.

When compared with α-amylase from *B. stearothermophilus* (ATCC 39709), the lyophilized preparation was shown to possess the same enzyme-specific activity, relative molecular mass, peptide maps, and reactivity towards antibody raised against α-amylase from *B. stearothermophilus* (ATCC 39709). This preparation produced no significant toxicological effects in a 13-week feeding study in dogs at levels of up to 0.20 g per kg of body weight per day, nor in a one-generation (one-litter) reproduction study in rats in which some of the offspring were treated at levels up to 0.50 g per kg of body weight per day for 14 weeks after weaning.

The Committee allocated an ADI "not specified" to this enzyme preparation.

A toxicological monograph and new tentative specifications were prepared.

α-Amylase from Bacillus megaterium expressed in Bacillus subtilis

α-Amylase produced extracellularly by the controlled fermentation of *Bacillus subtilis* B1-109 (ATCC 39701) containing the gene for α-amylase from *B. megaterium* (NCIB 11568) inserted by plasmid pCPC800 has not been previously evaluated by the Committee. At its present meeting, the Committee reviewed the available data pertaining to the genetic modification procedures employed, characterization of the producing organisms, the fermentation process, and acute, short-term, and reproduction studies with the lyophilized enzyme preparation.

The Committee noted that well-documented non-pathogenic and non-toxigenic strains of microorganisms had been employed in the genetic modification procedures. The plasmid construct pCPC800, containing both the α-amylase gene from *B. megaterium* and the promoter region of

the α-amylase gene from *B. stearothermophilus,* was introduced into *B. subtilis* (ATCC 39701) by standard transformation procedures. Data indicating the absence of antibiotic resistance, of production of "Shiga-like" toxin, and of infectivity potential of the α-amylase producing microorganism were provided.

The *B. subtilis* was grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation. The fermentation broth was filtered and the filtrate lyophilized before being mixed into the test diets. No viable cells or plasmid DNA could be detected in the amylase product.

The lyophilized preparation produced no significant toxicological effects in a 13-week study in dogs at levels of up to 0.57 g per kg of body weight per day, nor in a one-generation (one-litter) reproduction study in rats in which some of the offspring were treated at levels of up to 1.35 g per kg of body weight per day for 13 weeks after weaning.

The Committee allocated an ADI "not specified" to this enzyme preparation.

A toxicological monograph and new tentative specifications were prepared.

Chymosin A produced from Escherichia coli K-12 containing calf prochymosin A gene

Chymosin A produced from genetically modified *Escherichia coli* K-12 by fermentation has not been previously evaluated by the Committee. At its present meeting, the Committee reviewed the available data pertaining to the molecular construction of the chymosin expression plasmid, characterization of the producing organism, the fermentation process, biochemical and enzymic characterization of the recombinant enzyme, and short-term feeding studies on the enzyme product.

The Committee noted that a well-documented non-pathogenic strain of *E. coli* had been used as the host organism for the expression plasmid (pPFZ87A), which was derived from a widely used cloning vector (pBR322). The prochymosin A coding sequence, which was inserted into pBR322 plasmid, was initially synthesized chemically and shown to be identical to that of the natural calf gene. The plasmid construct was introduced into the host organism by standard transformation procedures, and the ampicillin-resistance gene carried by the plasmid was used as a marker for the selection of the transformed cells. Genetic stability of the transformed cells was demonstrated after repeated subculturing.

The transformed cells were grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation. Prochymosin A was recovered from the producing organisms after disruption and separation of the cells. Residual cells were inactivated by acidification, which also served to activate prochymosin A to chymosin A.

After chromatographic purification, the active enzyme was formulated with the stabilizer and preservatives typically used in commercial enzyme preparations.

The recombinant enzyme was extensively characterized and shown to be chemically and functionally identical to bovine chymosin A.

The results of studies on the microbiological purity of the enzyme product indicated the absence of producing organisms, minimal transfer of endotoxin from the bacterial cell walls, and the presence of insignificant levels of "Shiga-like" toxins. The low levels of residual DNA detected in the enzyme product consisted of short fragments without any demonstrable genetic activity.

The Committee noted that in a short-term (one-month) study in rats to which the enzyme product was administered, no adverse effects were observed at the highest dose level of 5 mg of chymosin per kg of body weight per day.

Taking into account the available safety information and the extremely low intake resulting from its use in food production, the Committee established an ADI "not specified" for the recombinant chymosin A preparation.

A toxicological monograph and new tentative specifications were prepared.

Chymosin B produced from Aspergillus niger var. awamori containing calf prochymosin B gene

Chymosin B produced from the genetically modified *Aspergillus niger* var. *awamori* by fermentation has not been previously evaluated by the Committee. At its present meeting, the Committee reviewed the available data pertaining to the molecular construction of the chymosin expression plasmid, characterization of the producing organism, the fermentation process, biochemical, immunological and enzymic characterization of the recombinant enzyme, and various toxicological studies on the enzyme product.

The host organism, GC delta AP4 strain of *A. niger* var. *awamori,* was derived from the parent strain NRRL3112 after a series of genetic manipulations. The host organism was auxotrophic and required uridine and arginine for growth; these properties served as selectable markers for subsequent genetic manipulations. The gene coding for aspergillopepsin A, an extracellular aspartic proteinase that degrades chymosin and causes "off" flavour in cheese, was also absent.

The expression plasmid (pGAMpR) was constructed on the basis of the pBR322 plasmid, a widely used cloning vector, and the prochymosin B gene sequence was obtained from calf stomach tissue.

The constructed expression plasmid was integrated into the host genome by standard transformation procedures. The potential pathogenicity of the resultant chymosin B-producing organism was studied in mice for 4 weeks

following the administration of a single oral dose. No evidence of pathogenicity was observed.

The cells were grown under properly controlled conditions in media containing ingredients commonly used in the production of food-grade substances by fermentation. The cells secreted chymosin B in its mature form, which was recovered from the fermentation broth by liquid–liquid extraction with polyethylene glycol, after removal of the cells. The resultant crude enzyme was further purified by chromatography. An alternative purification method was also used, in which the fermentation broth, after removal of the cells, was filtered through diatomaceous earth and the crude enzyme was further purified by chromatography. The purified chymosin B was formulated to the commercial strength with ingredients typically used in commercial enzyme preparations.

The recombinant enzyme was extensively characterized and shown to be enzymically and immunologically identical to calf chymosin B. The recombinant enzyme differed from calf chymosin B only in the degree of glycosylation, but was otherwise biochemically identical.

The chymosin B preparation was tested for other enzyme activities that might be present; in all cases, the activities were either not detected or detected at very low levels. Results from additional studies indicated the absence of mycotoxins, of antimicrobial activity, of residues of polyethylene glycol and of producing organisms.

In a short-term (90-day) feeding study in rats, no adverse effects were noted at levels up to 10 mg of chymosin preparation per kg of body weight per day. Negative results were also obtained in a series of standard mutagenicity and clastogenicity tests.

Taking into account the available safety information and the extremely low intake resulting from its use in food production, the Committee established an ADI "not specified" for the recombinant chymosin B preparation.

A toxicological monograph and new tentative specifications were prepared.

Chymosin B produced from Kluyveromyces lactis containing calf prochymosin B gene

Chymosin B produced from the genetically modified yeast *Kluyveromyces lactis* by fermentation has not been previously evaluated by the Committee. At its present meeting, the Committee reviewed the available data pertaining to the molecular construction of the chymosin expression plasmid, characterization of the producing organism, the fermentation process, biochemical and enzymic characterization of the recombinant enzyme, and various toxicological studies on the enzyme product.

The host organism for the chymosin expression plasmid was originally isolated from dairy products, and is a known source of the commercial lactase preparation. It is neither toxicogenic nor pathogenic for humans.

Plasmid pUC18 was used as the cloning vector for the prochymosin B coding sequence. The sequence was generated by a procedure involving several steps, starting with the isolation and purification of pre-prochymosin mRNA from the calf stomach. A complementary DNA (cDNA) sequence to the preprochymosin mRNA was prepared, cloned into plasmid pBR322, amplified in *Escherichia coli* as an intermediate host, and identified by DNA sequencing. The isolated prochymosin sequence was inserted into plasmid pUC18, and the expression plasmid was integrated into the host genome by standard transformation procedures. The transformed cells were identified by their resistance to G418 aminoglycoside.

The transformed yeast cells were cultured in medium containing food-grade substances commonly used in the fermentation process. During cultivation, prochymosin B was secreted into the medium. Fermentation was stopped by acid treatment, which also served to activate prochymosin B to chymosin B. After cellular debris was removed by filtration, the chymosin B-containing filtrate was further purified by several cell filtrations and concentrated by ultrafiltration. The enzyme concentrate was formulated with materials normally used in the cheese industry.

The recombinant enzyme was extensively characterized and shown to be biochemically, enzymically and immunologically identical to bovine chymosin B. The purity of the recombinant enzyme was reported to be much higher than that of rennet extract. There was no evidence of antimicrobial activity. In addition, there were no other proteases, detectable residues of recombinant DNA or viable yeast cells present in the purified enzyme preparation.

Various standard toxicological tests on the recombinant enzyme preparation were reviewed by the Committee. In an acute toxicity study in rats given a single oral dose of 5 g per kg of body weight of the enzyme preparation no evidence of toxicity was observed. In studies in rats in which cheese prepared with the recombinant chymosin was given in the diet at a level of 5 g per day for 21 or 91 days, no treatment-related effects were noted. In another study, incorporation of the recombinant enzyme preparation into the diet at dose levels up to 1 g per kg of body weight per day for 90 days did not cause any adverse effects in rats. *In vitro* genotoxicity studies indicated that recombinant chymosin B is not mutagenic.

On the basis of the available safety information and the extremely low exposure resulting from its use in food production, the Committee established an ADI "not specified" for the recombinant chymosin B preparation.

A toxicological monograph and new tentative specifications were prepared.

3.1.3 *Flavouring agents*

Allyl esters (allyl hexanoate, allyl heptanoate, and allyl isovalerate)

Temporary specifications for allyl hexanoate were prepared at the twenty-fourth meeting of the Committee (Annex 1, reference *53*), but it has not previously been evaluated by the Committee for the purpose of establishing an ADI. Allyl heptanoate and allyl isovalerate have not previously been considered by the Committee.

In evaluating these flavours, the Committee noted that they are rapidly hydrolysed to allyl alcohol and the corresponding acids by intestinal mucosal, pancreatic, and hepatic esterases. The results of studies on the toxicity of the three esters indicated that the hepatotoxicity observed at high doses was due to the allyl alcohol and its metabolites. Accordingly the Committee considered supplementary toxicological data on allyl alcohol and concluded that the three esters should be evaluated for a group ADI on the basis of the allyl alcohol moiety. In its evaluation, the Committee also took account of the principles relating to food flavours outlined in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*).

The hepatotoxicity of the esters and of allyl alcohol was less marked in repeated-dose short-term studies than in single-dose acute studies, although the mechanism of the acquired tolerance has not been fully elucidated. Mutagenicity studies on allyl hexanoate and allyl isovalerate yielded negative results, while most tests on allyl alcohol were negative.

The Committee reviewed two long-term carcinogenicity studies in rats and mice in which allyl isovalerate was administered by gavage in corn oil at both the maximum tolerated dose and 50% of this dose. Epithelial hyperplasia and squamous-cell papillomas of the forestomach were observed in mice, but not rats, at the highest dose. There was no evidence of hepatic tumours in mice (the liver being the target organ for short-term toxicity). The Committee concluded that these results were not relevant to the low-dose, dietary exposure to allyl isovalerate as a food flavour but were probably due to the effects of the large bolus doses that were used. The Committee also noted the small increase in the incidence of leukaemia reported in the treated rats; however, the incidence was within the historical control range and no increase in the incidence of hepatic tumours occurred in rats. Since levels of dietary exposure to allyl isovalerate in food are much lower than the doses used in these studies, the Committee concluded that an ADI could be set.

The evaluation was based on the no-observed-effect level in the short-term studies on allyl alcohol, with particular reference to hepatotoxicity; this provides a more conservative estimate than one based on the no-observed-effect level for the esters. The Committee noted that a number of other food flavours in use are allyl esters and should be considered for inclusion in the group ADI on the basis of their hydrolysis to allyl alcohol. In addition, in view of evidence that allyl esters of such fatty

acids as acetate, propionate, isobutyrate, and 2-ethylhexanoate are also rapidly hydrolysed, the Committee considered that their consumption should be taken into account since they could contribute to the total dietary load of allyl alcohol.

The Committee allocated an ADI of 0–0.05 mg per kg of body weight for allyl heptanoate, allyl hexanoate and allyl isovalerate as allyl alcohol equivalent. This corresponds to 0–0.15 mg per kg of body weight for allyl heptanoate, 0–0.13 mg per kg of body weight for allyl hexanoate, or 0–0.12 mg per kg of body weight for allyl isovalerate, or *pro rata* combinations of these substances.

A toxicological monograph was prepared. In addition, new tentative specifications for allyl heptanoate and allyl isovalerate were prepared. Information is required for both substances (see Annex 3). The existing tentative specifications for allyl hexanoate were revised and the "tentative" classification was retained because further information is required (see Annex 3).

trans-Anethole

trans-Anethole was first evaluated at the eleventh meeting of the Committee when a conditional ADI of 0–1.25 mg per kg of body weight was allocated (Annex 1, reference *14*). After re-evaluation at the twenty-third meeting (Annex 1, reference *50*), a temporary ADI of 0–2.5 mg per kg of body weight was allocated pending submission of the results of an adequate long-term study. After further reviews at the thirty-first and thirty-third meetings (Annex 1, references *77* and *83*), the Committee extended the temporary ADI but reduced it to 0–1.2 mg per kg of body weight pending further details of the long-term study and a review of the detailed study records and of the histological material.

At its present meeting, the Committee considered the results of three independent reviews of the liver histology in the long-term study in rats. It concluded that there is a clear increase in the incidences of hepatocellular adenomas and carcinomas in female rats at 10 mg/kg in the diet but not at lower doses. In male animals a slight increase in the incidence of hepatocellular adenomas but not carcinomas was observed at 10 mg/kg in the diet. There was also evidence of an increase in the incidence of non-neoplastic proliferative lesions in the liver at all dose levels in both sexes.

A review of the report of the long-term rat study showed no significant discrepancies. The Committee also noted that differences in survival between treated and control animals could not account for the increased incidence of liver tumours observed in the study.

The results of two new studies on genotoxicity (unscheduled DNA synthesis) were negative.

The Committee concluded that insufficient data were available to permit a final evaluation of the significance of the malignant liver tumours observed

in female rats with respect to the use of *trans*-anethole as a food additive. Further metabolic and especially pharmacokinetic studies in mice, rats, and humans are required for evaluation in 1992. In addition, a long-term dietary study in mice may be needed, although the design of such a study will depend upon the results of the metabolic and pharmacokinetic studies. In view of the positive results that were obtained in *in vitro* bacterial gene mutation tests, the Committee also concluded that chromosome aberration studies and *in vitro* tests for gene mutations in mammalian cells were desirable.

The temporary ADI was extended until 1992, but reduced to 0–0.6 mg per kg of body weight on the basis of the minimal effect level of 2.5 mg/kg in the diet (equivalent to a dose of 125 mg per kg of body weight per day) for non-neoplastic proliferative changes in the liver of rats, adjusted using a safety factor of 200.

The need for a reproduction/teratogenicity study will be considered by the Committee when further relevant data from the above studies have been reviewed. The Committee also reiterated that an epidemiological study of the effects of consuming high dietary levels of *trans*-anethole would be desirable.

A toxicological monograph was prepared. The existing specifications were maintained.

(+)-Carvone and (–)-carvone

(+)-Carvone and (–)-carvone were last evaluated by the Committee at its twenty-seventh meeting (Annex 1, reference *56*), when the temporary ADI of 0–1 mg per kg of body weight for the sum of the isomers was extended pending submission of the results from long-term studies in rats and mice with (+)-carvone.

At its present meeting, the Committee considered that optical enantiomers should not *per se* be regarded as toxicologically identical compounds, and therefore (+)-carvone and (–)-carvone should be evaluated separately. Although both compounds were placed on the agenda, sufficient data for toxicological evaluation had been submitted only for (+)-carvone.

In a long-term toxicity/carcinogenicity study in mice in which (+)-carvone was administered by gavage, no evidence of tumorigenicity was observed. Additional short-term studies in mice and rats, in which (+)-carvone was administered by gavage, and *in vitro* mutagenicity tests were also considered. Rats appear to be more susceptible to (+)-carvone than mice. The no-observed-effect level for (+)-carvone in rats was 93 mg per kg of body weight based on the study in which the substance was administered by gavage for 3 months. The Committee noted that, in an earlier 1-year feeding study in rats in which a no-effect level of 125 mg per kg of body weight per day was observed, it was not specified which isomer had been used.

The Committee noted that only limited data were available on the metabolism and pharmacokinetics of (+)-carvone.

An ADI of 0–1 mg per kg of body weight per day was established for (+)-carvone, based on a no-observed-effect level of 93 mg per kg of body weight per day in rats. The temporary ADI for (–)-carvone was not extended, because insufficient data were available for toxicological evaluation.

A toxicological monograph on carvone, including the limited available information on (–)-carvone, was prepared. The existing specifications for (+)-carvone and (–)-carvone were revised.

3.1.4 *Food colour*

Erythrosine

Erythrosine was last evaluated by the Committee at its thirty-third meeting (Annex 1, reference *83*), when a temporary ADI of 0–0.05 mg per kg of body weight was established. The evaluation was based on a no-observed-effect level with respect to thyroid function in humans ingesting a dose of 60 mg per day (equivalent to 1 mg per kg of body weight per day) for 14 days and a safety factor of 20. The Committee requested the results of pharmacokinetic studies that relate the level of absorption of erythrosine to the amount ingested.

At its present meeting, the Committee considered additional studies on thyroid hormone metabolism and regulation in male rats during 60-day feeding trials with erythrosine. The studies showed a rapid onset in the expected hormonal changes of a statistically significant rise in serum levels of thyrotropin, thyroxine (T_4) and 3,3′,5′-triiodothyronine (rT_3), and a decrease in 3,5,3′-triiodothyronine (T_3) after ingestion of 40 mg/kg erythrosine in the diet. A no-observed-effect level of 0.6 mg/kg erythrosine in the diet corresponding to 30 mg per kg of body weight per day was obtained. The changes seen in these studies are consistent with the hypothesis that erythrosine inhibits the hepatic conversion of circulating T_4 to T_3, and the resulting decrease in the concentration of T_3 stimulates the serial release of thyrotropin-releasing hormone from the hypothalamus and then thyrotropin from the pituitary. The sustained increases in the levels of thyrotropin produce hyperstimulation of the thyroid, which may be associated with the tumorigenic effects noted below.

The Committee also reconsidered the carcinogenicity data from two long-term feeding studies on erythrosine in which an increase in the incidence of thyroid follicular-cell adenomas in male rats was demonstrated at a level of 40 mg/kg of erythrosine in the diet. When thyroid follicular-cell adenomas and carcinomas were combined in the statistical analysis, significant (but not clearly dose-related) increases in the incidence of thyroid tumours in male rats given 1, 5, 10 and 40 mg/kg of erythrosine in the diet were found. Effects in females were significant only at one dose level. The Committee agreed that it was appropriate to

combine thyroid follicular-cell adenomas and carcinomas in the statistical analysis, in view of evidence that adenomas are an earlier stage of carcinomas in the thyroid.

The Committee reviewed additional data on the mutagenicity of erythrosine, and, taking into account extensive data from other mutagenicity studies, concluded that the compound is not genotoxic.

Results from the requested pharmacokinetic studies were not submitted. While clearly desirable and possibly helpful in defining no-effect levels, the Committee considered that such studies were unlikely to help in further clarifying the mechanism of the effect of erythrosine on thyroid function and of its tumorigenicity.

While a no-effect level could not be determined for the tumorigenic effect of erythrosine in rats, the Committee considered that the occurrence of thyroid tumours in rats was most likely secondary to hormonal effects and concluded that it would be possible to establish an ADI from the no-effect level for effects on thyroid function. In view of the differences in thyroid physiology between humans and rats the Committee based its evaluation on the previously reported no-observed-effect level derived from human data. Therefore the Committee allocated an ADI of 0–0.1 mg per kg of body weight for erythrosine, based on the no-observed-effect level of 60 mg per person per day (equivalent to 1 mg per kg of body weight per day) and a safety factor of 10.

An addendum to the toxicological monograph was prepared. The existing specifications for erythrosine were revised.

3.1.5 *Sweetening agents*

Acesulfame potassium

This substance was last considered by the Committee in 1983 at its twenty-seventh meeting (Annex 1, reference *62*), when it was concluded that there was no evidence that the substance was mutagenic or carcinogenic. In long-term (2-year) feeding studies in rats and dogs, the no-observed-effect level for acesulfame potassium was 30 mg/kg in the diet (equivalent to 1.5 g per kg of body weight per day in rats and equal to 900 mg per kg of body weight per day in dogs). At that time the Committee allocated an ADI of 0–9 mg per kg of body weight based on the dog study, in which the no-observed-effect level was the highest dose tested, and a safety factor of 100 (Annex 1, reference *63*).

At its present meeting, the Committee reviewed further data which confirmed the validity of the earlier long-term study in rats and the no-observed-effect level. A review of comparative pharmacokinetic data in rats and dogs showed that the blood levels of acesulfame potassium reached after similar doses were higher in dogs; there was no evidence to suggest that in relation to blood levels, the dog was more sensitive to the effects of the substance than the rat.

Pharmacokinetic studies in humans showed that oral doses of acesulfame potassium were completely absorbed and rapidly excreted unchanged in the urine. The half-life in the plasma was 1.5 hours, which indicated that the period of exposure to the substance was brief and no accumulation occurred.

Since acesulfame potassium was not metabolized in any species tested, including humans, and further studies in rats in which repeated doses were given did not reveal any induction of metabolism or change in pharmacokinetic behaviour, the Committee concluded that the rat appeared to be an appropriate model for humans. Consequently, the Committee decided that, since the 2-year study in rats represented a greater proportion of the lifespan of the species than did the 2-year study in dogs and included exposure to the substance *in utero,* the ADI should be based on the no-observed-effect level in the rat, i.e. 1500 mg per kg of body weight per day. The Committee also noted new data which indicated that acesulfame potassium had no adverse effects in diabetic rats and was not allergenic in an active systemic anaphylaxis test in guinea-pigs.

The Committee also reviewed extensive toxicological studies on the breakdown products acetoacetamide and acetoacetamide-*N*-sulfonic acid, which indicated that these compounds have a low toxicity and are not mutagenic.

In view of these data and of available estimates of levels of exposure to acesulfame potassium, the Committee concluded that acetoacetamide-*N*-sulfonic acid and acetoacetamide did not represent a health hazard under present or foreseeable conditions of use of acesulfame potassium.

The previously established ADI was changed to 0–15 mg per kg of body weight based on the long-term study in rats.

A toxicological monograph was prepared.

The Committee recommended that the specifications should be reviewed and revised in the near future, taking into account current manufacturing and purification procedures.

Trichlorogalactosucrose (TGS)

Trichlorogalactosucrose (TGS) was previously evaluated at the thirty-third meeting of the Committee (Annex 1, reference *83*), when a temporary ADI of 0–3.5 mg per kg of body weight was allocated, based on the no-observed-effect level of 750 mg per kg of body weight per day in a 1-year study in dogs and a safety factor of 200. At that time, the Committee requested: (a) information on the absorption and metabolism of TGS in humans after prolonged oral dosing; (b) results of studies to ensure that TGS produces no adverse effects in people with insulin-dependent and maturity-onset diabetes; (c) results of further studies in rats on the elimination of TGS from pregnant animals and from the fetus, to exclude the possibility of bioaccumulation; and (d) results of a short-term rat study on 6-chlorofructose.

At its present meeting, the Committee reviewed new and previously available data. Although no new data on the absorption and metabolism of TGS in humans were received, the Committee concluded that there was no indication that these processes would change on prolonged oral dosing. This conclusion was drawn from the comparative metabolic data for TGS in various species, including humans, and the lack of evidence of toxicity in extensive animal studies. Nevertheless, the Committee recognized that the data did not address all possibilities, particularly the potential effects of adaptation of the gastrointestinal microflora.

No specific studies on possible adverse effects of TGS in people with insulin-dependent and maturity-onset diabetes had been performed. However, the Committee decided that this concern could be satisfactorily addressed through consideration of data which showed that TGS had no effect on the secretion of insulin in humans or rats, blood glucose levels or carbohydrate metabolism. Furthermore, the Committee was aware of proposed studies involving both types of diabetics.

On re-assessment of the overall data on TGS, including the metabolic data in various species, including humans and pregnant and non-pregnant rabbits, and in the absence of any significant finding in the two-generation reproduction study in rats, the Committee concluded that the question on the accumulation of TGS in pregnant animals and fetuses was satisfactorily addressed, and that there was no evidence to suggest a difference in metabolism in pregnant and non-pregnant animals.

The Committee reviewed additional studies relating to the possible toxicity of the potential breakdown product of TGS, 6-chlorofructose. In a short-term study (28 days) in which 6-chlorofructose was administered at 240 and 480 mg per kg of body weight per day, male mice showed hind-limb paralysis. In addition three special studies were conducted to assess reproductive function in rats. Administration of 6-chlorofructose at 18–48 mg per kg of body weight per day for 7–14 days caused a loss of fertility in male rats. In two of these studies, the no-effect levels were 3 and 6 mg per kg of body weight per day. The Committee noted, however, that 6-chlorofructose is only a potential breakdown product of TGS. While a hypothetical maximum exposure of 1.15 µg per kg of body weight per day to humans would occur if TGS were subjected to extreme conditions, such as 0.1 mol/l HCl at 68 °C for 72 hours, the Committee expected that exposure to 6-chlorofructose would be virtually nil under all foreseeable storage or physiological conditions.

Finally, the Committee concluded that since the 2-year study in rats, which included a period of exposure to TGS *in utero,* represented a greater proportion of the lifetime of the species than did the 1-year study in dogs, the former study should be used for the purposes of setting an ADI. A safety factor of 100 was therefore applied to the no-observed-effect level in the long-term study in rats (1500 mg per kg of body weight per day), and an ADI of 0–15 mg per kg of body weight was allocated.

Additional immunotoxicity studies to assess the significance of observed weight changes in the spleen and thymus and changes in lymphocyte counts in rats were considered to be desirable. As yet, a causal relationship between these findings and high levels of exposure to TGS cannot be excluded.

An addendum to the toxicological monograph was prepared.

The Committee received a request to reconsider the title "trichlorogalactosucrose" it had adopted at its thirty-third meeting (Annex 1, reference *83*) and to rename it "sucralose". At that time, the Committee established guidelines for designating titles for specifications monographs and selected the title in accordance with those guidelines. The Committee considered the information submitted at the present meeting and concluded that criteria 1 (names established by international organizations) and 2 (names established by governmental legislation) were not applicable. It did not believe that the information submitted in relation to criterion 3 (names established by common usage) was sufficiently compelling to change the name, and reaffirmed its view, reached in relation to criterion 4 (available scientific, common or trivial names), that "trichlorogalactosucrose" was appropriate. In this regard, the Committee noted that the name "sucralose" had been designated as a synonym in the specifications monograph. The existing tentative specifications were revised and the Committee agreed to delete the "tentative" classification.

3.1.6 *Miscellaneous food additives*

Dimethyldicarbonate (DMDC)

Dimethyldicarbonate (DMDC) has not been previously evaluated by the Committee. It has a broad antimicrobial spectrum and is used as a cold sterilization agent for fruit juices, soft drinks, and wines. DMDC is unstable in aqueous solutions and breaks down almost immediately after addition to beverages. The principal breakdown products in wine and aqueous liquids are methanol and carbon dioxide. Dimethylcarbonate and methylethylcarbonate, as well as carbomethoxy adducts of amines, sugars, and fruit acids, are also formed in small amounts. In the presence of traces of ammonia or ammonium ions (e.g., in wines), DMDC forms trace quantities of methylcarbamate.

The Committee reviewed data from acute toxicity studies with DMDC in mice and rats, as well as short- and long-term toxicity studies in rats that received juices and alcoholic beverages that had been treated with 4 g/l of DMDC, and a 1-year toxicity study in dogs. Data from reproduction toxicity, embryotoxicity/teratogenicity, and genotoxicity studies with DMDC-treated beverages were also examined. It was concluded that there was no evidence of toxic effects in mice and rats due to the consumption of DMDC-treated beverages.

The Committee also reviewed data from acute toxicity studies with methylethylcarbonate, dimethylcarbonate, and several carboxymethylation

products of amino and hydroxy acids, as well as short-term toxicity studies in rats with methylethylcarbonate and dimethylcarbonate, and an embryotoxicity/teratogenicity study in rats with methylethylcarbonate. No adverse effects due to the consumption of these decomposition products were observed.

In the case of methylcarbamate, the Committee reviewed data from acute toxicity studies in mice and rats, short-term studies in mice and rats, long-term carcinogenicity studies in mice and rats, dermal carcinogenicity and DNA-binding studies in mice, a large number of studies on genotoxicity in bacterial and mammalian cells (including *in vivo* studies), and a special study on immunotoxicity in mice. Methylcarbamate produced hepatocellular carcinomas in Fischer 344 rats at high dose levels, but did not have such effects in Wistar rats or in mice. Methylcarbamate was shown to be non-genotoxic.

The no-observed-effect level for hepatic carcinogenesis in Fischer 344 rats was 100 mg per kg of body weight per day. Since the estimated worst-case exposure of humans to methylcarbamate in beverages would be less than 20 µg/l at the concentrations of DMDC employed, a large margin of safety applies. The Committee concluded, therefore, that the presence of methylcarbamate at the expected levels of use of DMDC (i.e., in accordance with Good Manufacturing Practice) would not be of risk to human health.

The concentrations of methanol (up to 120 mg /l) resulting from the use of DMDC are similar to or less than those occurring naturally in many fruit juices and alcoholic beverages. The Committee considered that the concentrations of methanol present after treatment of beverages with DMDC were of no toxicological concern.

DMDC was considered acceptable for use as a cold sterilization agent for beverages when used in accordance with Good Manufacturing Practice up to a maximum concentration of 250 mg/l.

A toxicological monograph and new specifications for dimethyl-dicarbonate were prepared.

Dioctyl sodium sulfosuccinate (DSS)

Dioctyl sodium sulfosuccinate (DSS) was previously reviewed at the eighteenth, twenty-second and twenty-fourth meetings of the Committee (Annex 1, references *35*, *47* and *53*).

At its twenty-second meeting, the Committee withdrew the temporary ADI of 0–2.5 mg per kg of body weight because the additional information requested at its eighteenth meeting had not been provided. The required information included: (a) the effects on newborn animals, particularly those exposed to the substance through lactation; (b) an adequate long-term study in a rodent species; and (c) an investigation of pulmonary circulatory effects, including pulmonary hypertension. These data were still not available at the twenty-fourth meeting.

Since the previous evaluation, additional toxicological data have become available, and these were reviewed by the Committee at its present meeting.

The Committee noted that results from a three-generation reproduction study with DSS in rats did not reveal any adverse effects on the reproductive function of either sex at dose levels up to 10 g/kg in the diet. Neither was there any evidence of adverse effects on the offspring as a result of prenatal and postnatal exposure to DSS. However, DSS did cause a reduction in parental body weight as well as weanling pup weight at dose levels of 5 g/kg and above in the diet. It was concluded that the no-observed-effect level of DSS was 1 g/kg in the diet, equivalent to 50 mg per kg of body weight per day.

In a long-term study, DSS did not exhibit tumour promotional activity in rats pre-exposed to a model gastrointestinal carcinogen. However, a full carcinogenicity bioassay that meets modern standards is still lacking.

Two inhalation studies in which rabbits and dogs were exposed to DSS were reviewed by the Committee. No adverse pulmonary or systemic effects were indicated from the results.

The Committee based its evaluation of DSS on the no-observed-effect level found in the three-generation reproduction study in rats, to which a safety factor of 200 was applied. A temporary ADI of 0–0.25 mg per kg of body weight was allocated to DSS, pending the evaluation, in 1995, of the results of the long-term study in a rodent species that was requested earlier.

A toxicological monograph was prepared. The existing specifications were revised.

Gellan gum

Gellan gum, an extracellular polysaccharide produced from *Pseudomonas elodea* by fermentation, was evaluated for the first time by the Committee. The substance has a high relative molecular mass and is used as a stabilizer and thickening agent in foods.

P. elodea is an aerobic, Gram-negative bacterium, which has been very well characterized and demonstrated to be non-pathogenic.

Gellan gum was shown to be poorly absorbed and did not cause any deaths in rats which received a single large dose (5 g per kg of body weight) in the diet or by gavage. Short-term (90-day) exposure of rats to gellan gum at levels up to 60 g/kg in the diet did not cause any adverse effects. In a 28-day study in prepubertal monkeys, no overt signs of toxicity were observed at the highest dose level of 3 g per kg of body weight per day. In reproduction and teratogenicity studies in rats in which gellan gum was given at dose levels up to 50 g/kg in the diet, there was no evidence of interference with the reproductive process, and no embryotoxic or developmental effects were observed. Gellan gum was also shown to be non-genotoxic in a battery of standard short-term tests.

In a study in dogs, which were treated for 1 year at dose levels up to 60 g/kg in the diet, there were no adverse effects that could be attributed to chronic exposure to gellan gum. In long-term carcinogenicity studies, gellan gum did not induce any adverse effects in mice or rats at the highest dose levels of 30 g/kg and 50 g/kg in the diet, respectively.

Results from a limited study on tolerance to gellan gum in humans indicated that oral doses of up to 200 mg per kg of body weight administered over a 23-day period did not elicit any adverse reactions, although faecal bulking effects were observed in most subjects.

The Committee allocated an ADI "not specified" to gellan gum, and pointed out that its potential laxative effect at high intakes should be taken into account when it is used as a food additive (Annex 1, reference *88*, section 2.2.3).

A toxicological monograph and new specifications for gellan gum were prepared. Microbiological criteria were specified that took into account the nature of the gum and the manufacturing procedure.

Mineral oil (food-grade)

Food-grade mineral oil was last evaluated by the Committee at its thirty-third meeting (Annex 1, reference *83*).

At its present meeting, the Committee reconsidered two 90-day feeding studies in rats, which received both oleum-treated and hydrogenated mineral oils. In the first study, haematological changes and deposition of mineral oil in the liver, spleen and lymph nodes were reported; in the second, only deposition in the liver, spleen and lymph nodes was reported, but no haematological investigations were conducted.

The Committee was unable to determine whether the deposition of these oils in the liver, spleen and lymph nodes was of toxicological significance. However, it considered the haematological findings to be toxicologically significant. Both effects require further investigation.

The Committee was aware of proposals to conduct short-term feeding studies in rats with several different mineral oils in which these effects would be investigated, and stated that it wished to review these studies when they became available.

In view of the uncertainties raised by the 90-day studies submitted and the lack of long-term feeding studies, the Committee decided that an adequate long-term feeding study should be performed using food-grade mineral oils representative of those in commercial use.

The Committee extended the current temporary ADI "not specified" for mineral oil until 1995, when the results of such studies should be made available for evaluation.

A toxicological monograph was not prepared.

At its thirty-third meeting, the Committee had been concerned about the lack of adequate data on the chemical characterization of the food-grade hydrogenated mineral oils (Annex 1, reference *83*), and had requested further data on the chemical composition of both mineral oils currently in use and on mineral oils used in previous toxicological studies, in order to characterize these materials adequately. The requested information was received by the Committee at its present meeting. The existing tentative specifications were revised and the "tentative" qualification was deleted.

3.2 Contaminants

3.2.1 *Benzo[a]pyrene*

Benzo[*a*]pyrene has not been previously evaluated by the Committee. The Committee noted that the Codex Committee for Food Additives and Contaminants had requested the evaluation of the impact on human health of benzo[*a*]pyrene when present as a food contaminant.

Although benzo[*a*]pyrene was the substance on the agenda, the Committee recognized that this was only one member of a class of more than 100 compounds belonging to the family of polycyclic aromatic hydrocarbons found in food and that they should be considered as a class.

The Committee reviewed and discussed data from studies on the toxicity of benzo[*a*]pyrene, with particular emphasis on its toxicity after ingestion. The results demonstrated many different toxic effects of this compound.

In mice, orally administered benzo[*a*]pyrene consistently produced tumours of the forestomach and lung, and the few available studies in rats showed tumours of the oesophagus, forestomach and mammary gland. Tumours at other sites, such as lymphoreticular tumours in mice, were also reported. The Committee noted that fetal and newborn mice are especially vulnerable to the pulmonary and lymphatic tumorigenicity of benzo[*a*]pyrene administered either by direct injection or transplacentally.

The genotoxicity of benzo[*a*]pyrene, both *in vitro* and *in vivo,* is well documented, and this compound is consequently used as a positive control substance in these types of studies. The Committee noted that IARC had found inadequate evidence for carcinogenicity of benzo[*a*]pyrene in humans, but sufficient evidence for carcinogenicity in animals and for activity in short-term genotoxicity tests.

The Committee also noted that in mice oral doses of 120 mg of benzo[*a*]pyrene per kg of body weight and above caused intrauterine toxic effects and fetal malformations when administered during pregnancy.

The immunotoxicity and bone-marrow toxicity of benzo[*a*]pyrene in mice were also considered. In the immunotoxicity studies, in which pregnant mice received a single dose of benzo[*a*]pyrene at 150 mg per kg of body weight intraperitoneally, the resultant offspring were severely immuno-suppressed. The Committee noted that this effect may have led to the subsequent widespread development of tumours in these animals.

Many studies have implicated benzo[*a*]pyrene-7,8-diol-9,10-oxide (BPDEI) as the proximate carcinogenic metabolite of benzo[*a*]pyrene. This metabolite binds covalently to DNA, induces mutations and transformations in short-term tests, and is a highly potent carcinogen in mouse skin. However, the Committee noted that studies in which benzo[*a*]pyrene was administered perorally to mice and rabbits showed that the level of binding of BPDEI to DNA was similar in all tissues examined (target tissues as well as non-target tissues).

The Committee also considered studies in which the levels of BPDEI-DNA adducts were measured in human tissues, although none of these studies were aimed at monitoring benzo[*a*]pyrene exposure from food. The Committee noted that the levels of DNA adducts were elevated in only a few individuals, who were believed to have been exposed to high levels of benzo[*a*]pyrene. The levels of BPDEI-DNA adducts in humans were unrelated to factors such as age, sex, ethnicity, number of cigarettes smoked, or caffeine consumption.

It was concluded that, for the purpose of evaluation, the most significant toxicological effect of benzo[*a*]pyrene was its carcinogenic activity.

The Committee had before it data from studies on benzo[*a*]pyrene levels in various foods and estimated dietary intakes. These data demonstrated the wide-ranging levels of benzo[*a*]pyrene in food and that these levels were dependent on factors such as where the food was grown (i.e., industrialized or non-industrialized area), how it was processed (e.g., smoking or drying), and how it was cooked (e.g., charcoal grilling). In turn, dietary intakes varied considerably, some consumers being exposed to high levels of the substance.

The Committee noted that the estimated average daily intake of benzo[*a*]pyrene by humans was about four orders of magnitude lower than the level reported to be without effect on the incidence of tumours in an experiment in rats in which benzo[*a*]pyrene was incorporated in the diet. However, the Committee was unable to establish a tolerable intake for benzo[*a*]pyrene, based on the available data.

Nevertheless, the large difference between estimated human intakes of benzo[*a*]pyrene and the doses producing tumours in animals suggests that any effects on human health are likely to be small. Despite this, the considerable uncertainties in risk estimation require that efforts should be made to minimize human exposure to benzo[*a*]pyrene as far as is practicable.

The Committee was informed that a long-term carcinigenicity study in rats in which benzo[*a*]pyrene is being administered by gavage has been initiated to investigate the dose–response relationship for the tumorigenicity of this compound.

The Committee acknowledged the complexities of the problem of reducing exposure to benzo[*a*]pyrene and other polycyclic aromatic

hydrocarbons. Furthermore, it noted that exposure to benzo[*a*]pyrene constitutes only a fraction of consumers' exposure to polycyclic aromatic hydrocarbons, and that some other members of this class of compounds, not evaluated at this meeting, have similar toxicological properties to benzo[*a*]pyrene and may thus contribute to the overall carcinogenic risk. In this regard, strategies to minimize exposure to benzo[*a*]pyrene would also be effective in reducing overall exposure to polycyclic aromatic hydrocarbons. These include practices that consumers can effect, such as washing fruits and vegetables thoroughly to remove any surface contamination and, prior to barbecuing meats, trimming excess fat to minimize "flare-ups" and cooking in a fashion that prevents contact of the food with flames. Measures that can be taken by the food industry include conversion to indirect heating for drying foods, switching to non-coal-fired roasters (e.g., for roasting coffee beans), using protective coverings (e.g., cellulose casing) when smoking foods conventionally, and ensuring compliance with limits for polycyclic aromatic hydrocarbons in food additives specified by national or international bodies. The Committee urged the application of these measures to minimize contamination of food with polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene.

A toxicological monograph was prepared.

3.2.2 *Ochratoxin A*

Ochratoxin A has not been previously evaluated by the Committee. The Committee noted that several species of *Aspergillus* and *Penicillium* are capable of producing ochratoxin A. This mycotoxin occurs naturally in human food and animal feedstuffs.

The Committee reviewed studies on the metabolic disposition and toxicology of ochratoxin A, as well as limited information on the association between exposure to ochratoxin A and chronic human nephropathy, endemic in Bulgaria, Romania, and Yugoslavia.

Metabolic studies indicated that ochratoxin A is mainly absorbed from the proximal jejunum and stomach. Absorption varied from 40% to 60% and serum half-life ranged from 4 to > 500 hours, depending on the species. In blood, ochratoxin A was predominantly bound to serum albumin and other unidentified macromolecules. Tissue concentrations of ochratoxin A residues followed the order kidney > liver > muscle > fat. Ochratoxin A was excreted via the urine and faeces. In cattle and sheep, ochratoxin A was largely hydrolysed to the relatively non-toxic ochratoxin α.

The underlying mechanism of the toxic action of ochratoxin A is believed to be specific competitive inhibition of phenylalanine-tRNA ligase (phenylalanyl-tRNA synthetase).

Acute toxicity studies indicated that the pig and dog were the most sensitive species and that death was due to widespread multifocal haemorrhage, intravascular coagulation, and necrosis of the liver, kidney and lymphoid organs. Short-term studies in rats, dogs and pigs showed that

the dominant pathological effects occurred in the kidneys. Progressive nephropathy was observed in each species, characterized by a deterioration in kidney function and, histologically, by karyomegaly and necrosis of the tubular cells, and thickening of the tubular basement membranes. The severity of the effects depended on the dose and sensitivity of the animal species used. Long-term studies in mice and rats demonstrated that, in addition to nephropathy, there was a dose-related increase in the incidence of benign and malignant tumours. Rats appeared to be more sensitive than mice. The majority of the genotoxicity tests on ochratoxin A were negative.

Ochratoxin A also exhibited teratogenic activity in rats and mice, with the central nervous system being the predominant target tissue.

In experimental animals treated with ochratoxin A, both humoral and cell-mediated immunity as well as structural components of the immune system were adversely affected.

The effects of ochratoxin A that were considered to be most significant by the Committee are summarized in Table 1. The kidney appeared to be the primary target organ and the most sensitive species was the pig. As no-observed-effect levels were frequently not demonstrated and since the effects were observed in a small proportion of the pig's lifetime, the

Table 1
Summary of effects observed in laboratory animal studies, following oral administration of ochratoxin A

Effect	Species	Duration of treatment	Lowest-observed-effect level (mg per kg of body weight per day)	No-observed-effect level (mg per kg of body weight per day)
Deterioration in renal function	Pig	90 days	0.008	—[a]
Karyomegaly of the proximal tubular cells	Rat	90 days	0.015	—[a]
Progressive nephropathy	Pig	2 years	0.04	0.008
Overt fetal craniofacial anomalies	Mouse	—[b]	1	—[a]
Kidney tumours	Mouse	2 years	4.4	0.13
	Rat	2 years	0.07	0.02
Necrosis of lymphoid tissues of thymus and tonsils	Dog	14 days	0.1	—[a]
Decreased antibody response	Mouse	50 days	—[c]	0.5

[a] No-observed-effect levels were not demonstrated in these studies.
[b] Results refer to a teratogenicity study in which ochratoxin A was administered on day 9 of gestation.
[c] Only one dose level was used.

Committee concluded that, in assessing the tolerable intake of ochratoxin A, a 500-fold margin of safety should be applied to the lowest-observed-effect level of 0.008 mg per kg of body weight per day. On this basis, a provisional tolerable weekly intake of 112 ng per kg of body weight was established.

Chronic human nephropathy, endemic in some areas of the Balkans, has been linked with exposure to ochratoxin A, as indicated by the presence of ochratoxin A residues in local foodstuffs as well as in the blood of inhabitants. On the other hand, some individuals and village populations have had detectable ochratoxin A residues in the blood, but have shown no evidence of nephropathy. This suggests that either the effects of ochratoxin A are delayed or the disease is caused by more than one factor. About one-third of those dying with Balkan endemic nephropathy have had papillomas and/or carcinomas of the renal pelvis, ureter or bladder. No quantitative estimates of ochratoxin A dietary intake were available.

Data on the occurrence of ochratoxin A have demonstrated significant levels in a variety of foods although the overall incidence of positive samples is low. As a result, it is extremely difficult to estimate total dietary exposure to ochratoxin A for the general population, although worst-case intakes of the order of 1 to 5 ng per kg of body weight per day have been estimated in populations where there is no evidence of nephropathy.

The Committee was informed that the occurrence of elevated ochratoxin A levels in foodstuffs in areas with endemic nephropathy was associated with poor conditions for grain storage; this factor has been recognized as being important in the production of ochratoxin A.

The Committee therefore recommended that efforts be made to highlight the need for instituting proper storage conditions for grain and grain products. Furthermore, monitoring of appropriate ochratoxin A residues should be undertaken to obtain better estimates of dietary exposure and to identify populations at greatest risk with a view to implementing preventive measures. The Committee also encouraged further studies to elucidate the role of ochratoxin A and other mycotoxins in nephropathy in pigs and humans, the mechanism of induction of tumours, and the role of phenylalanine in antagonizing the adverse effects of ochratoxin A.

A toxicological monograph was prepared.

4. Revision of certain specifications

4.1. General

Thirteen substances were evaluated for specifications only (see Annex 2), and the specifications for twelve were revised. The existing tentative specifications for the remaining substance, citrus red no. 2, were

withdrawn because the Committee had concluded at its thirteenth meeting that this substance should not be used as a food colour (Annex 1, reference *19*).

Information was received indicating that the name "carbon black" was often used as a synonym for "channel black", a colouring agent not used in food, and was therefore potentially misleading. The Committee decided to change the name of the food colour to "vegetable carbon". The specifications were revised and the "tentative" qualification (Annex 1, reference *79*) was deleted. The Committee also received substantive comments on the existing specifications for "activated carbon" and revised the specifications accordingly.

For two substances, diethyl ether and mixed carotenoids, some of the information requested by the Committee (Annex 1, references *64* and *77*) had not been received. The Committee therefore revised the existing tentative specifications for both, and maintained the "tentative" qualifications.

The Committee considered six substances with existing tentative specifications (disodium and tetrasodium pyrophosphates, glycerol ester of wood rosin, lecithin, partially hydrolysed lecithin and sucrose acetate isobutyrate) and concluded that sufficient information was now available to revise these specifications and to delete the "tentative" qualifications.

The specifications for polydimethylsiloxane were revised to include a wider range of viscosities, which reflects recent changes to the commercial product.

The existing tentative specifications for xanthan gum were revised in accordance with the changes to the microbiological criteria. It was decided not to specify the criteria for specific organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Xanthomonas campestris,* as the manufacturing process would preclude their contamination of the product.

4.2 Addendum to the general specifications for enzymes used in food processing

At its thirty-fifth meeting (Annex 1, reference *88*), the Committee prepared revised general specifications for enzyme preparations used in food processing. These were subsequently published in FAO Food and Nutrition Paper No. 49 as an annex (Annex 1, reference *90*).

At its present meeting, the Committee recognized that these general specifications were not sufficient to address new concerns relating to enzyme preparations from genetically modified organisms. The Committee therefore prepared a draft addendum to these general specifications, which will be published by FAO in the forthcoming revised *Guide to specifications.* The addendum is titled "General considerations and specifications for enzymes from genetically manipulated microorganisms" and is considered "tentative" pending comment from

interested parties and future review and possible revision by the Committee.

5. Future work

1. During its evaluation of specifications, the Committee noted that most of the current limits for trace impurities, such as lead and arsenic, had remained for many years without revision. It therefore recommended that such limits be re-examined (with a view to their being lowered) to ensure that they reflect current manufacturing practices for food additives. As part of this exercise, consideration should be given to specifying trace impurities of relevance to particular additives and to the need to revise existing methods for their determination.
2. The Committee was advised that all existing specifications established by the Committee were being assembled into a single compendium, to be published by FAO. It was noted that many of the older specifications were not in accordance with current standards. The Committee concluded that, as time and the agenda of future meetings permit, these specifications should be reviewed and revised to reflect current requirements.
3. In view of the potential for confusion between enzyme preparations from a growing number of conventional and genetically modified microbial sources, guidelines should be developed to ensure a consistent approach to nomenclature.

6. Recommendations

1. In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, and the increased number of requests from the Codex Committee on Food Additives and Contaminants, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives should continue to be held at least once yearly to evaluate these substances.
2. A large number of food colours have been evaluated at various meetings of the Committee. Commercial preparations often contain a high proportion of impurities, which may cause toxicological effects not characteristic of the pure colour. The Committee concluded that safety data should therefore be generated using the commercial product. In addition, industry should be encouraged to produce colours of a greater degree of purity so as to simplify both safety evaluations and the preparation of specifications.
3. Manufacturers should be encouraged to deposit newly developed strains of microorganisms that are used in the production of enzyme preparations and other food additives with national and international culture collections. The strains would then be assigned unique

registration numbers to facilitate their identification. Furthermore, FAO and WHO should encourage international cooperation in the development of such culture collections and gene banks, to facilitate the identification of genetically manipulated microorganisms.

4. During its evaluation of mineral oil, the Committee noted that, in the specifications for this substance, the content of polycyclic aromatic hydrocarbons was determined by a nonspecific limit test that measured these hydrocarbons as a group. The Committee concluded that more precise information was needed on the levels and nature of individual polycyclic aromatic hydrocarbons in mineral oils that were subject to toxicity testing. It also recommended that information on other possible impurities be obtained for future safety evaluations of this food additive.
5. During its evaluation of the contaminant benzo[*a*]pyrene, the Committee noted that this compound is a member of the class of substances known as polycyclic aromatic hydrocarbons, and is only one of the many such hydrocarbons to which humans are exposed. The Committee therefore recommended that any future evaluations consider polycyclic aromatic hydrocarbons as a class. A similar approach is recommended when evaluating other substances that belong to a chemically related group, including food additives such as the allyl esters considered at the present meeting.
6. Efforts should be made to minimize human exposure to benzo[*a*]pyrene and ochratoxin A.
7. FAO and WHO, in cooperation with other international organizations, should encourage the development of improved multi-residue methods (to detect two or more polycyclic aromatic hydrocarbons, in the µg/kg range) as well as techniques to confirm the identity and amount of individual polycyclic aromatic hydrocarbons, also in the µg/kg range.
8. Ochratoxin A has been shown to be transmitted from feed to animal tissues. Efforts to destroy ochratoxin A in feed grains using ammonia have not proved satisfactory, and thus studies on the detoxification of ochratoxin A in contaminated feed grains should be continued.
9. The Committee is continually faced with the problem of assessing the relevance of very low levels of carcinogenic chemicals occurring as contaminants. Therefore the Committee recommends that potentially carcinogenic compounds in food be identified, in order to collect data on their occurrence, the influence of food technology, and toxicology, so as to set priorities for the purpose of assessing the safety of these compounds.

Acknowledgement

The Expert Committee wishes to thank Professor P. Shubik, Green College, Oxford, England, for his valuable contribution to the meeting.

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 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 international 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 (out of print).
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 (out of print).
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 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No.40A, B, C, 1967; 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 of the Expert Committee). FAO Nutrition Meetings Report Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

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. 45A, 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, 1970; 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, 1970; 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 (out of print).
33. *Toxicological evaluation of certain food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers.* FAO Food and Nutrition Paper, No. 4, 1978.
35. *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.
36. *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.
37. *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.
38. *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.
39. *Toxicological evaluation of some food colours, thickening agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
40. *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.
41. *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.
42. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives.* FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. *Summary of toxicological data of certain food additives.* WHO Food Additives Series, No. 12, 1977.
46. *Specifications for identity and purity of some food additives, including*

antioxidants, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. *Summary of toxicological data of certain food additives and contaminants.* WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives.* FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives.* FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).* FAO Food and Nutrition Paper, No. 17, 1980.
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Annex 2

Acceptable Daily Intakes, other toxicological information, and information on specifications

Food additive	Specifications[1]	Acceptable Daily Intake (ADI) and other toxicological recommendations
Antioxidants		
Butylated hydroxytoluene (BHT)	R	0–0.125 mg per kg of body weight[2]
tert-Butylhydroquinone (TBHQ)	R	0–0.2 mg per kg of body weight[2]
Erythorbic acid and its sodium salt[3]	R[4]	ADI not specified[5]
Enzyme preparations		
α-Amylase from *Bacillus stearothermophilus*	N	ADI not specified[5]
α-Amylase from *Bacillus subtilis*	N	ADI not specified[5]
α-Amylase from *Bacillus stearothermophilus* expressed in *Bacillus subtilis*	N, T	ADI not specified[5]
α-Amylase from *Bacillus megaterium* expressed in *Bacillus subtilis*	N, T	ADI not specified[5]
Chymosin A produced from *Escherichia coli* K-12 containing calf prochymosin A gene	N, T	ADI not specified[5]
Chymosin B produced from *Aspergillus niger* var. *awamori* containing calf prochymosin B gene	N, T	ADI not specified[5]
Chymosin B produced from *Kluyveromyces lactis* containing calf prochymosin B gene	N, T	ADI not specified[5]
Flavouring agents		
Allyl heptanoate	N, T	0–0.15 mg per kg of body weight[6]
Allyl hexanoate	R, T	0–0.13 mg per kg of body weight[6]
Allyl isovalerate	N, T	0–0.12 mg per kg of body weight[6]
trans-Anethole	S	0–0.6 mg per kg of body weight[2]
(+)-Carvone	R	0–1 mg per kg of body weight
(–)-Carvone	R	No ADI allocated[7]

Food additive	Specifications[1]	Acceptable Daily Intake (ADI) and other toxicological recommendations
Food colour		
Erythrosine	R	0–0.1 mg per kg of body weight
Sweetening agents		
Acesulfame potassium	S	0–15 mg per kg of body weight
Trichlorogalactosucrose	R	0–15 mg per kg of body weight
Miscellaneous food additives		
Dimethyldicarbonate	N	Acceptable[8]
Dioctyl sodium sulfosuccinate	R	0–0.25 mg per kg of body weight[2]
Gellan gum	N	ADI not specified[5, 9]
Mineral oil (food-grade)	R	ADI not specified[2, 5]

Contaminant	Provisional Tolerable Weekly Intake (PTWI)
Benzo[*a*]pyrene	PTWI not established[10]
Ochratoxin A	112 ng per kg of body weight

Food additive	Specifications only[1]
Activated carbon	R
Citrus red no. 2	W
Diethyl ether	R, T
Disodium pyrophosphate	R
Glycerol ester of wood rosin	R
Lecithin	R
Lecithin, partially hydrolysed	R
Mixed carotenoids	R, T
Polydimethylsiloxane	R
Sucrose acetate isobutyrate	R
Tetrasodium pyrophosphate	R
Vegetable carbon	R[11]
Xanthan gum	R

Notes to Annex 2

1. N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or not required; T, the existing, new, or revised specifications are tentative and comments are invited; and W, existing specifications were withdrawn (see Annex 3).

2. Temporary acceptance (see Annex 3).
3. This substance was on the agenda under the name "isoascorbic acid".
4. Existing specifications were revised for erythorbic acid only; specifications for the sodium salt were not reviewed.
5. ADI "not specified" means that, on the basis of the available data (chemical, biochemical, toxicological, and other), the total daily intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary.
6. The listed values correspond to an allyl alcohol equivalent of 0.05 mg per kg of body weight, which is the group ADI for the listed allyl esters. They may be used alone or in *pro rata* combinations in such a way that the intake of allyl alcohol does not exceed this value.
7. The previous temporary ADI was not extended.
8. Acceptable for use as a cold sterilization agent in beverages when used according to Good Manufacturing Practice up to a maximum concentration of 250 mg/l.
9. The potential laxative effect of gellan gum at high intakes should be taken into account when it is used as a food additive.
10. Insufficient information available to establish a PTWI.
11. Supersedes the earlier tentative specifications for carbon black published in FAO Food and Nutrition Paper, No. 38, 1988.

Annex 3

Further toxicological studies and other information required or desired

Antioxidants

Butylated hydroxytoluene (BHT)

The final results of the ongoing study on the development and role of hepatic changes in long-term toxicity in male Wistar rats after exposure to BHT *in utero* are required for evaluation in 1994.

tert-Butylhydroquinone (TBHQ)

1. The results of the ongoing long-term toxicity studies in rodents are required for evaluation in 1994.
2. Additional data on genotoxicity of TBHQ, obtained by current techniques, are desirable.

Flavouring agents

Allyl heptanoate

Information is required on:
- solubility and identification by infrared spectrum,
- solubility in ethanol, and analytical methods to determine the ethanol content.

Allyl hexanoate

A method for determining the allyl alcohol content is required.

Allyl isovalerate

Information is required on:
- solubility and identification by infrared spectrum,
- solubility in ethanol, analytical methods to determine the allyl alcohol content and assay procedures to determine allyl isovalerate.

trans-Anethole

The results of further metabolic and pharmacokinetic studies in mice, rats, and humans are required for evaluation in 1992.

Submission of the results of chromosome aberration studies and *in vitro* tests for gene mutations in mammalian cells, and of an epidemiological study of the effects of consuming high dietary levels of *trans*-anethole is desirable.

Food colour

Erythrosine

Submission of the results of pharmacokinetic studies relating the amount of erythrosine absorbed to the amount ingested is desirable.

Sweetening agents

Trichlorogalactosucrose

Submission of the results of immunotoxicity studies to assess the significance of observed weight changes in the spleen and thymus and changes in lymphocyte counts in rats is desirable.

Miscellaneous food additives

Dioctyl sodium sulfosuccinate

The results of a long-term study in a rodent species are required for evaluation in 1995.

Mineral oil (food-grade)

1. The results of an adequate long-term feeding study using food-grade mineral oils representative of those in commercial use are required for evaluation in 1995.
2. Submission of the results of planned short-term feeding studies with several different mineral oils is desirable.

Specifications only

Diethyl ether

Information is required on the nature and levels of stabilizers used and their methods of analysis.

Mixed carotenoids

Information is required on the composition of the commercial product and analytical methods to distinguish between mixed carotenoids and synthetic colours.

Annex 4

Matters of interest arising from meetings of the Codex Alimentarius Commission and the Codex Committee on Food Additives and Contaminants

Matters arising from the Eighteenth Session of the Codex Alimentarius Commission

The Codex Alimentarius Commission, at its Eighteenth Session in July 1989 (ALINORM 89/40), considered a paper entitled "Implications of biotechnology on international food standards and codes of practice" (ALINORM 89/39). This paper addressed general issues that may arise in the Codex system's consideration of the products of biotechnology, including (a) the role of new technologies in food production; (b) the effect of biotechnology on existing Codex definitions; (c) the safety evaluation of foods, food ingredients, and food additives developed through biotechnology; and (d) the designation of substances derived by biotechnology on the label of food products. The Codex Alimentarius Commission recommended that this paper be brought to the attention of the Joint FAO/WHO Expert Committee on Food Additives for comments.

The Expert Committee noted that many people viewed recent developments in biotechnology, such as recombinant DNA and transgenic techniques, and the products of these technologies with suspicion. In consideration of such attitudes among consumers, the Codex Alimentarius Commission might wish to introduce its paper with a preamble to address these anxieties, offering assurance that the Codex Alimentarius Commission will exercise an appropriate degree of caution when considering these products.

The Committee wished to point out that one of its roles was to evaluate the safety of products of biotechnology in food and noted that several such products had been considered at its present meeting.

Matters arising from the Twenty-second Session of the Codex Committee on Food Additives and Contaminants

1. The Expert Committee was informed about progress in adopting a possible new approach to the safety evaluation of food additives. This approach was based on a paper entitled "Future activities of the Committee in regard to the establishment and regular review of provisions relating to food additives in Codex Standards and possible mechanisms for the establishment of general provisions for the use of food additives in non-standardized foods" (CX/FAC/89/16).

   The paper analysed the use of food additives and the role of the Codex Committee in dealing with them. A major problem that was identified was that the Codex Committee deals only with standardized foods

(which have gone through the Codex procedure) and does not set limits for the use of additives in these foods in a very detailed manner, while most foods are non-standardized. In this regard, the Codex Alimentarius Commission is trying to adopt a more "horizontal" approach to food additives, which would lead to a Codex Standard for food additives instead of a Codex Standard for commercial products only.

In considering whether to adopt the procedure outlined in this paper, the Codex Committee decided to concentrate on two groups of food additives, antioxidants and preservatives. The Codex Committee agreed that it should have sufficient information from the Expert Committee on how to translate ADIs into levels of use in food and drinks. If further information was required, the Codex Committee would seek assistance from the Expert Committee by means of a comprehensive list of clear direct questions.

The Codex Commitee agreed to confine itself to the list of substances that have been evaluated by the Expert Committee for the time being, but noted that there are many other food additives that may require evaluation.

In the paper on the general provisions for the use of food additives, it was also stated that a full understanding of the basis for ADIs is necessary and the Codex Committee therefore stressed that the Expert Committee should explain its evaluations as clearly as possible.

If this new approach is taken, there may be a need for additional meetings of the Expert Committee. The frequency of such meetings ultimately depends upon the resources that are made available for this purpose. At its present meeting, the Expert Committee indicated its willingness to be as responsive as possible to the Codex Committee and its commitment to explain its evaluations clearly.

2. The Expert Committee was informed that the International Numbering System (INS) and the definitions of titles of classes of food additives had been finalized by the Codex Committee and would be submitted to the Codex Alimentarius Commission for adoption.

   The INS is intended to be a system for identifying food additives approved for use in Codex member countries. It does not imply approval by Codex but is a means of identifying food additives used worldwide and includes many additives additional to those that have been given ADIs by the Expert Committee.

   The Expert Committee took note of this information. Action was not required.

3. The Codex Committee agreed with the proposal, by the Secretariat of the Expert Committee, for a procedure regarding the future review of new specifications prepared by the Expert Committee and the publication of Codex Advisory Specifications.

4. The Expert Committee was informed about the growing activities of the Codex Committee concerning environmental contaminants. The approach taken by the Codex Committee to contaminants differs from its approach to food additives, since the presence of food additives is controlled, while the presence of contaminants in food is unintentional.

   The Codex Committee requested information from governments regarding their national strategies towards environmental contaminants, including the setting of guideline levels or maximum levels. When necessary, the Codex Alimentarius Commission will work on harmonization of these levels.

   At its present meeting, the Expert Committee evaluated the contaminant benzo[*a*]pyrene. As noted in section 3.2.1, the Expert Committee believed that it would be more appropriate to evaluate the polycyclic aromatic hydrocarbons as a class rather than to investigate individual contaminants. Therefore, it was recommended that, whenever feasible, the Codex Committee refer classes of contaminants rather than individual chemicals in the class to the Expert Committee for review.

5. The Codex Committee had prepared an inventory of compounds to be evaluated by the Expert Committee. However, no priorities had been set for the safety review of these compounds and, because of the large number of substances on the list, the Expert Committee recommended that this task be carried out at the next session of the Codex Committee.

6. The Codex Committee expressed concern about the frequency of future meetings of the Expert Committee. Although three meetings of the Expert Committee were being held in the 1990–91 biennium, only one would be devoted to the evaluation of food additives and contaminants. This issue would be discussed at the Twenty-third Session of the Codex Committee as well as at the Conference on Food Standards, Chemicals in Food, and Food Trade, in March 1991.

Annex 5

Matters of interest arising from the Forty-third World Health Assembly

The Forty-third World Health Assembly, in May 1990, adopted a resolution on "Prevention and control of iodine deficiency disorders" in which the Joint FAO/WHO Expert Committee on Food Additives was asked to "verify the effectiveness and safety of the long-term use of potassium iodide and potassium iodate to fortify salt for the prevention and control of iodine deficiency disorders".

Iodine is an essential trace element, but iodine deficiency continues to be a public health problem in many countries.

The Committee noted that over the past 50 years many countries in Europe, the Americas, Oceania and Asia have successfully eliminated or have made significant progress in the control of iodine deficiency disorders, largely through the iodization of salt with potassium iodide or potassium iodate, assisted by a diversification of the diet.

The Committee drew attention to the Provisional Maximum Tolerable Daily Intake (PMTDI) for iodine of 1 mg from all sources, which was established at the thirty-third meeting (Annex 1, reference *83*). At its ninth meeting (Annex 1, reference *11*), the Committee had concluded that the use of potassium iodate as a flour treatment agent was unacceptable because the resultant intake of iodine would be excessive. However, this was not considered relevant to the use of potassium iodate as a source of dietary iodine, subject to the PMTDI not being exceeded.

Potassium iodate and potassium iodide have a long-standing and widespread history of use for fortifying salt without apparent adverse health effects. The Committee noted that potassium iodate has been shown to be a more suitable substance for fortifying salt than potassium iodide, because of its greater stability, particularly in warm, damp or tropical climates. In addition no data are available indicating toxicological hazard from the ingestion of these salts below the level of the Provisional Maximum Tolerable Daily Intake.

The Committee therefore concluded that potassium iodate and potassium iodide should continue to be used for this important public health purpose.

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