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EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Forty-fourth report of the
Joint FAO/WHO Expert Committee on
Food Additives

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Joint FAO/WHO Expert Committee on Food Additives

Rome, 14–23 February 1995

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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. 35, in preparation.

Specifications are issued separately by FAO under the title:

Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.

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 Rome from 14 to 23 February 1995. The meeting was opened by Dr H. de Haen, Assistant Director-General, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr de Haen noted that the Committee had a unique scientific reputation and standing in the international community. Members of the Committee, invited in their personal capacities, provided advice and opinion which was respected by all Member States of FAO and WHO. The scientific integrity and independence of the Committee were its greatest assets. Dr de Haen cited one of the recommendations of the Joint FAO/WHO Conference on Food Standards, Chemicals in Food and Food Trade held in cooperation with GATT in 1991 (*1*), namely that the Committee should only consider questions of health and safety and technical concerns, and should not become involved in social, ethical or similar matters that should be addressed in other forums.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*2*), there have been forty-three previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the forty-first meeting (Annex 1, reference *107*).

The tasks before the Committee were:

(a) to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
(b) to undertake toxicological evaluations of certain food additives and contaminants (section 3 and Annexes 2 and 3); and
(c) to review and prepare specifications for selected food additives (sections 3 and 4 and Annexes 2 and 3).

2.1 Modification of the agenda

Nitrate and nitrite had been included in the agenda as contaminants. In evaluating these substances (and the potential endogenous formation of *N*-nitroso compounds) (see section 3.2.2), the Committee considered all sources of intake, including that resulting from food additive use and from their presence as constituents of certain foods, especially vegetables.

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 Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*), as well as principles elaborated subsequently at meetings of the Committee (Annex 1, references *77, 83, 88, 94, 101* and *107*), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference *76*) embraces the major observations, comments and recommendations on the safety assessment of food additives and contaminants contained, up to the time of its publication, in the 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 *Safety evaluation of flavouring agents*

The Committee considered a paper prepared for the meeting by Dr I.C. Munro outlining an approach to the safety evaluation of flavouring agents which incorporates a series of criteria designed to provide a means of evaluating such agents in a consistent and timely manner. The criteria, which have been drawn up in the light of the principles for the safety evaluation of flavouring substances (Annex 1, reference *76*), take account of available information on intake from current uses, structure-activity relationships, and metabolism and toxicity data. They incorporate procedures outlined in the report of the thirty-third meeting of the Committee (Annex 1, reference *83*), which include a method for dividing flavouring substances up into three structural classes based on structural characteristics and metabolism. The use of these criteria provides a means of ranking flavouring substances in terms of concern over potential inherent toxicity and provides guidance on the nature and extent of the data required to perform a safety evaluation. The details of the evaluation scheme and the supporting scientific documentation are discussed in the background document that will be published with the toxicological monographs from the meeting (see page viii). The evaluation criteria are briefly discussed below.

The criteria take advantage of the fact that some flavouring agents occur as normal constituents of mammalian tissues or are metabolized to form such constituents, and are then completely metabolized to innocuous products such as carbon dioxide and water. It is proposed that flavouring agents having these characteristics should be considered safe for consumption if human intake is low, but should be evaluated on the basis of toxicity data if human intake is high. The safety evaluation may involve the use of toxicity data on the individual substance concerned or may rely, at least in part, on toxicity data on substances of closely related structure.

For flavouring agents that are not known to be metabolized to innocuous end-products, the safety evaluation must be based on toxicity data, even if intake is low. In such cases human intake of the flavouring agent must

be less than one-thousandth of the no-observed-effect level (NOEL) for the substance or the NOEL for a substance of related structure on which the safety evaluation relies.

For those flavouring agents currently in use for which no toxicity or metabolic data exist, but where intake is extremely low, it might be possible to specify a threshold below which intake is considered safe (human exposure threshold).

In considering the approach described above, the Committee noted that the evaluation of intake data on flavouring substances as described in the paper involved the comparison of intake with the human exposure thresholds for each of the three structural classes. These thresholds are derived from a reference database of NOELs which characterize the relationship between human intake and toxicity for a large number of substances of different chemical structure. The human exposure thresholds for each of the three structural classes provide a means of assessing the potential toxicity of a flavouring agent in relation to the intake. The Committee considered that it would be desirable to examine the effect that changing these thresholds would have on the outcome of the evaluations. It also noted that the intake estimates, as presented in the report, were based on data derived from surveys conducted in the United States of annual production figures for individual flavouring substances intended for use in food. It was noted that intake data may vary from one country to another and that the use even of conservative production figures may lead to the underestimation of the intake of certain flavouring agents. The Committee recommended that, when this procedure was used, intake data should be carefully documented, especially for substances that are widely used.

The key role of metabolic data in safety evaluation was discussed, and it was recommended that performance criteria for use in establishing standards for the conduct of metabolic studies should be considered.

The Committee concluded, as it had in the past, that the safety evaluation of flavouring agents warranted special consideration in the light of the large number of substances requiring evaluation and the fact that, for the majority of flavouring agents, human intakes are relatively low and self-limiting. A key new element in the proposed scheme is the use of human exposure thresholds as a means of evaluating the potential for toxicity in relation to intake. The integration of data on intake, in relation to the thresholds, with information on structure-activity relationships, metabolism and toxicity in the form presented in the criteria provides a means of conducting evaluations consistent with principles previously elaborated by the Committee.

The Committee recommended that, at a future meeting, the procedure should be applied to the evaluation of a number of flavouring agents belonging to different chemical classes in order to assess its utility in practice, taking into account the effect of varying the human exposure thresholds and in the light of a wider range of sources of intake data.

2.2.2 *Expression of Acceptable Daily Intakes*

At its thirty-sixth meeting, when residues of veterinary drugs in food were considered, the Committee decided that, in future, figures for Acceptable Daily Intakes (ADIs) would be given to only one significant figure (Annex 1, reference *91*). When an ADI is calculated from a NOEL that is given to more than one significant figure, it would therefore be rounded off to one significant figure, in accordance with accepted procedures.

At its present meeting, the Committee confirmed that this procedure would be extended to ADIs for food additives and tolerable intakes for contaminants.

2.2.3 *Terminology*

Many mechanisms, including a direct interaction with DNA, may be involved in the complex process leading to the emergence of tumours. However, the details of this process are not known (*3*). The Committee therefore concluded that it was not appropriate to use terminology that implies a mechanism in which genotoxicity is linked to carcinogenicity. In future, the Committee will describe a substance that has been determined to be both genotoxic and carcinogenic as a genotoxin and a carcinogen, rather than as a genotoxic carcinogen.

2.3 Principles governing the establishment and revision of specifications

2.3.1 *Flavouring agents*

The Committee decided that specifications would be established for flavouring agents as and when these substances are considered as described in section 2.2.1 and are placed on the agenda. Such specifications should take into account, in addition to the information and data submitted by FAO/WHO Member States, research institutes, manufacturers and individual scientists, the work already done by the Scientific Committee for Food of the European Union, the Committee of Experts on Flavouring Substances of the Council of Europe, and other international expert bodies and associations.

2.3.2 *Limits for arsenic, lead and heavy metals*

In response to a request made at the Twenty-fourth Session of the Codex Committee on Food Additives and Contaminants by its Working Group on Specifications (*4*), the Committee, at its forty-first meeting (Annex 1, reference *107*), recommended that future submissions on specifications should include actual concentrations of lead and certain other heavy metals found for substances under consideration. Since the reaction to this recommendation has not been encouraging, the Committee will consider at its future meetings lowering the existing general limits

of 10 mg/kg for lead and 40 mg/kg for heavy metals for substances under consideration unless data in support of these limits are provided.

In future evaluations the Committee will also assess, on a case-by-case basis, the need for limits for arsenic in specifications for substances under review. Such limits will be reduced or withdrawn unless the information provided, the nature and source of the substance, or the levels of consumption indicate that limits for arsenic are necessary.

2.3.3 *Microbiological criteria*

Work on the revision of the specifications for alginic acid (and its ammonium, calcium, potassium and sodium salts), processed *Eucheuma* seaweed, and gum arabic highlighted the need for consistency in allocating limits for microorganisms in food additives from natural sources. The Committee therefore requested that future submissions on specifications for such substances include information on their microbiological status (e.g. total aerobic plate counts and total numbers of moulds and yeasts, coliforms, and salmonellae) to enable it to establish suitable microbiological criteria where needed.

3. Comments on specific substances

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

3.1 Specific food additives

3.1.1 *Antioxidants*

Butylated hydroxytoluene (BHT)

Butylated hydroxytoluene (BHT) was previously evaluated by the Committee at its sixth, eighth, ninth, seventeenth, twentieth, twenty-fourth, twenty-seventh, thirtieth and thirty-seventh meetings (Annex 1, references *6, 8, 11, 32, 41, 53, 62, 73* and *94*). At the thirty-seventh meeting, the temporary ADI of 0–0.125 mg per kg of body weight, established at the previous meeting, was extended pending the results of a study designed to elucidate the role of hepatic changes in the development of hepatic carcinomas observed in male Wistar rats following *in utero* and lifetime exposure to BHT.

The results of the above study were reviewed at the present meeting. In addition, new data relating to the previously noted effects of BHT on the lung, liver, kidney, clotting mechanisms and promotion/inhibition of carcinogenesis, new long-term and reproductive toxicity studies,

genotoxicity assays and human observations were reviewed. The conclusions of these studies were consistent with the results of previous toxicity studies on BHT.

The effects of long-term BHT administration have been adequately documented in a number of rodent studies, in only one of which, namely that reported in 1986 and conducted in the Wistar rat, was a hepatocarcinogenic effect evident. This study differed from those conducted previously in that the rats were exposed to BHT *in utero*, during the lactation period, and for a further 40 weeks after the standard 2-year exposure period. The dose levels employed were 25, 100 or 500 mg per kg of body weight per day in the diet, except during the long-term feeding portion of the study, when the highest dose was reduced to 250 mg per kg of body weight per day because of the severity of hepatotoxic effects in the parental generation. A statistically significant increase in the survival-adjusted incidence of hepatocellular neoplasms was observed in both male and female rats at the highest dose tested. The majority of these tumours were not malignant; however, the incidence of hepatocarcinomas was significantly higher in male rats in the highest-dose group than in untreated males. The tumours were detected very late in the study, in most cases when the animals were killed (following 141–144 weeks of treatment). The NOEL was 25 mg per kg of body weight per day, based on effects on litter size, sex ratio, and pup body-weight gain during the lactation period in the reproduction segment of the study.

The Committee was aware that the International Agency for Research on Cancer had reviewed the above study (*5*), and had been unable to draw conclusions about the observed incidences of liver lesions in the treated groups because of the large differences in survival between treatment and control groups. The carcinogenicity of BHT to humans could not be evaluated.

The protocol employed in the new study, the purpose of which was to investigate the hepatic changes in male Wistar rats that occur following *in utero* and lifetime exposure to BHT for up to 22 months, was almost identical to that of the 1986 study. In the new study, hepatomegaly was observed in the F_0 dams receiving the highest dose (500 mg per kg of body weight per day), while toxicologically significant liver enlargement was not observed in any F_1 dose group up to 250 mg per kg of body weight per day, the highest dose tested. The body weights of the pups from the highest-dose group were significantly lower than those of control pups throughout the lactation period, and mortality was increased in the treated pups in a dose-related manner between days 6 and 21 of lactation. In the F_1 males, BHT administration resulted in a persistent, marked induction of cytochrome P-450 2B and γ-glutamyl transpeptidase activity in the centrilobular and periportal hepatocytes in the highest-dose group throughout the study, commencing at a very early stage (21 days of age); γ-glutamyl transpeptidase activity was also increased, but to a lesser extent, at the middle dose (cytochrome P-450

2B activity was not determined). Total cytochrome P-450 content and epoxide hydrolase, ethoxyresorufin *O*-deethylase and glutathione *S*-transferase activities were also consistently elevated in a dose-related manner in the mid- and highest-dose groups. (In a separate study, uridine diphosphate (UDP)-glucuronosyl transferase activity was induced in the liver of male Wistar rats receiving BHT in the feed at a dose level of 5 g/kg, equivalent to 500 mg per kg of body weight per day.) Consistent enlargement of the centrilobular hepatocytes was evident starting at 6 months in rats receiving the highest dose of 250 mg per kg of body weight per day, indicative of proliferation of the smooth endoplasmic reticulum consistent with the induction of mixed-function oxidases. However, histopathological examination failed to reveal any signs of hepatocellular necrosis in this group. In addition, no evidence of hepatotoxicity, as indicated by a decrease in glucose-6-phosphatase activity and intracellular glutathione content in the treated groups, was seen. There was no indication of sustained hepatocellular proliferation during the study. A small, but significant, number of altered hepatic foci deficient in glucose-6-phosphatase were found in the highest-dose group (250 mg per kg of body weight per day) at 22 months. In this same group, lesions described as hepatic nodules were detected in 6/19 animals as compared with none in the lower-dose groups and controls. Evidence of thyroid enlargement with follicular hyperplasia, in the absence of elevated serum thyroxine levels, was noted in the groups receiving 100 and 250 mg per kg of body weight per day. There was evidence in the mid- and highest-dose groups of an early, transient effect on the adrenal cortex before sexual maturation of the F_1 males, which took the form of cytomegaly of the cells of the zona fasciculata.

BHT has been shown to induce hepatocellular necrosis and proliferation in male Wistar rats at doses higher than those used in either of these long-term studies and which exceeded the maximum tolerated dose. Sublethal oral doses of 1000 or 1250 mg per kg of body weight per day for 4 days induced hepatocellular necrosis in the centrilobular region within 48 hours. When a lower dose of BHT (500 mg per kg of body weight per day) was administered for periods of 1–4 weeks, allowing for enzyme induction to occur, bile duct proliferation, hepatocellular hyperplasia, and persistent fibrous and inflammatory cell reactions were observed in the periportal region. The shift in the localization of the damage from one area of the liver to another suggested the involvement of inducible hepatic drug-metabolizing enzymes in the production of reactive metabolites.

In view of the probable involvement of hepatic enzyme induction in the development of the hepatocellular damage associated with repeated doses of BHT, the Committee concluded that, in this case, enzyme induction was the most sensitive index of effects on the liver. A well defined threshold was demonstrated at 100 mg per kg of body weight per day in the new long-term study reviewed for the first time at this meeting, giving a NOEL of 25 mg per kg of body weight per day. Effects observed

in the reproduction segments of the *in utero*/lifetime exposure studies were also taken into account in the derivation of this NOEL. The Committee used a safety factor of 100 to allocate an ADI of 0–0.3 mg per kg of body weight for BHT.

A toxicological monograph containing relevant studies from the previous monographs and monograph addenda and those reviewed for the first time at the present meeting was prepared. The existing specifications were maintained.

tert-Butylhydroquinone (TBHQ)

tert-Butylhydroquinone (TBHQ) was previously evaluated by the Committee at the nineteenth, twenty-first, thirtieth and thirty-seventh meetings (Annex 1, references *38, 44, 73* and *94*). At the thirty-seventh meeting, the previously established temporary ADI of 0–0.2 mg per kg of body weight was extended, pending receipt of the results of ongoing long-term toxicity studies in rodents. This ADI was derived from a NOEL of 1500 mg/kg of feed (equivalent to 37.5 mg per kg of body weight per day) in a 117-week feeding study in dogs on the basis of haematological abnormalities observed at the next highest dose level of 5000 mg/kg of feed. In addition, the Committee requested clarification of the results of genotoxicity assays available at that time, which indicated that TBHQ was clastogenic in both *in vitro* and *in vivo* assays, but was apparently devoid of activity in bacterial mutagenicity assays. At its present meeting, the Committee reviewed the results of all available genotoxicity assays with TBHQ, in addition to some new studies on its metabolism and disposition, its effects on the renal pelvic epithelium, and its role in the promotion/inhibition of cancer. The final results of long-term studies in mice and rats were not available for review.

TBHQ was shown to be oxidatively metabolized to *tert*-butylquinone both enzymatically and by autoxidation. The results of a number of studies indicated that *tert*-butylquinone participates in redox cycling through the formation of a semiquinone radical, such cycling being accompanied by the production of reactive oxygen species. Glutathione conjugates of *tert*-butylquinone were also shown to have the potential to participate in redox cycling reactions, suggesting that reactive oxygen species might be generated even after covalent binding of *tert*-butylquinone to cellular thiols.

The results of bacterial mutagenicity studies available to the Committee were uniformly negative in both the presence and the absence of a rat liver metabolic activation system, as observed previously. In mammalian cell mutagenicity assays, TBHQ was positive in one study at the thymidine kinase (TK) locus and negative in two studies at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus. It was noted that the TK locus is responsive to reactive oxygen species and can respond to clastogens, while the HGPRT locus is considerably less sensitive. Clastogenicity *in vitro* was demonstrated in all four

independent assays and *in vivo* in one of two independent studies. Micronuclei were induced in mouse bone marrow in one of two *in vivo* studies.

TBHQ caused DNA damage *in vitro* as a result of the formation of reactive oxygen species, but did not do so in the rat forestomach after administration by gavage. *tert*-Butylquinone, the oxidation product of TBHQ previously mentioned, did cause DNA damage in this system at a non-cytotoxic concentration. In this context, it has been shown that *tert*-butylquinone is produced in the rat forestomach following the administration of butylated hydroxyanisole (BHA), TBHQ being formed as an intermediate.

The Committee extended the temporary ADI of 0–0.2 mg per kg of body weight. The final results of the long-term toxicity studies in mice and rats that are known to have been completed are required for review in 1997.

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

3.1.2 ***Carrier solvent***

Diethylene glycol monoethyl ether

This compound was previously evaluated at the twentieth and thirty-ninth meetings of the Committee (Annex 1, references *41* and *101*), but no ADI was allocated because of inadequacies in the information available on long-term toxicity and carcinogenicity.

At its thirty-ninth meeting, the Committee was informed that the use of diethylene glycol monoethyl ether as a carrier solvent for flavours could lead to carry-over levels as high as 1000 mg per kg in foods as consumed. The toxicity data available on diethylene glycol monoethyl ether were derived from 90-day studies in mice, rats and pigs, genotoxicity assays and reproductive toxicity/teratogenicity studies. At that meeting, the Committee concluded that, in order to re-evaluate diethylene glycol monoethyl ether, it would require either:

(a) adequate data indicating that human intakes are sufficiently low for the principles applicable to materials occurring in foods in small amounts to apply; or
(b) the results of adequate carcinogenicity/chronic studies in rats and mice.

No relevant new toxicological data on diethylene glycol monoethyl ether were available at the present meeting. However, revised intake information was provided, based on the specific use of this substance as a carrier solvent in beverages containing citrus oil-based flavourings, from which the Committee concluded that the estimated intake of diethylene glycol monoethyl ether (15 mg per person per day or 0.25 mg per kg of body weight per day) was significantly lower than previously assumed. Nevertheless, this revised intake estimate was still too high to permit its evaluation as a material occurring in food in small amounts.

In view of the apparent potential for significant intake of diethylene glycol monoethyl ether and the absence of adequate long-term feeding studies, an ADI could not be allocated.

A toxicological monograph was not prepared. The existing specifications were maintained.

3.1.3 *Colour stabilizer*

4-Hexylresorcinol

This substance is used as a processing aid to prevent the development of melanosis (black spot) in shrimps and related crustacea, and was evaluated for this specific use only. It had not previously been evaluated by the Committee.

4-Hexylresorcinol is applied as an aqueous solution at concentrations of 50 mg/l, resulting in residue levels in the edible portion of crustacea of approximately 1 mg/kg. The estimated intake of the compound resulting from this use was 1–8 µg per person per day. Against this background, the Committee evaluated 4-hexylresorcinol on the basis of previously established principles for substances consumed in small amounts. The Committee was informed that throat lozenges containing 2.4–4 mg per lozenge of 4-hexylresorcinol for use as an oral antiseptic are available over the counter in some countries.

The Committee noted that toxicological information on this substance was limited, but recent 13-week and 2-year carcinogenicity studies in mice and rats were available. Nephrotoxicity was reported in mice in both the 13-week and 2-year carcinogenicity studies, female mice being affected at all dose levels. In the 2-year carcinogenicity study in mice, the lowest dose tested, 63 mg per kg of body weight per day, caused a high incidence of nephropathy. Nephrotoxicity was not reported in rats and 4-hexylresorcinol was not carcinogenic in either mice or rats.

On the basis of the available data in animals, the Committee was unable to establish a numerical ADI for 4-hexylresorcinol, but concluded that the treatment of crustacea at concentrations of up to 50 mg/l, resulting in residue levels of approximately 1 mg/kg in the edible portion, was not of toxicological concern.

The Committee emphasized that, for more extensive use or higher levels of application, further toxicological data would be required, including a long-term toxicity study in mice that establishes a clear NOEL and the results of reproductive toxicity/teratogenicity studies.

A toxicological monograph and new specifications were prepared.

3.1.4 *Emulsifiers*

Dioctyl sodium sulfosuccinate

Dioctyl sodium sulfosuccinate was previously reviewed at the eighteenth,

twenty-second, twenty-fourth and thirty-seventh meetings of the Committee (Annex 1, references *35, 47, 53* and *94*).

At its thirty-seventh meeting, the Committee evaluated several studies that had become available, including a 3-generation reproductive toxicity study in rats and two inhalation studies in rabbits and dogs. The NOEL in the reproductive toxicity study was equivalent to 50 mg per kg of body weight per day. The Committee concluded that previous concerns about the reproductive and pulmonary circulatory effects of dioctyl sodium sulfosuccinate had been satisfactorily addressed by these studies. A temporary ADI of 0–0.25 mg per kg of body weight was therefore established for dioctyl sodium sulfosuccinate, based on a safety factor of 200 and the NOEL in the reproductive toxicity study. The ADI was made temporary pending the receipt and evaluation of the results of a long-term toxicity study in a rodent species, which had been previously requested by the Committee at its twenty-second meeting (Annex 1, reference *47*).

Current estimates of the dietary intake of dioctyl sodium sulfosuccinate from food additive uses may reach 2 µg per person per day (equivalent to 0.03 µg per kg of body weight). Short-term use of the substance as a laxative and as an excipient in vitamin and mineral supplements can result in an exposure of up to 100 mg per person per day.

The Committee concluded that dioctyl sodium sulfosuccinate could be evaluated on the basis of the limited data available because of the low intake anticipated from food additive uses, and consequently withdrew its request for a long-term study. However, because of the limited toxicological database on dioctyl sodium sulfosuccinate, the Committee increased the previous safety factor of 200 to 500. On the basis of a NOEL of 50 mg per kg of body weight from the reproductive study in rats and the new safety factor of 500, the Committee established an ADI of 0–0.1 mg per kg of body weight for the food additive use of dioctyl sodium sulfosuccinate.

A toxicological monograph was not prepared. The existing specifications were maintained.

Glycerol ester of wood rosin

Glycerol ester of wood rosin was previously considered by the Committee at its eighteenth, twentieth and thirty-third meetings (Annex 1, references *35, 41* and *83*). At its twentieth meeting, the Committee, in the light of the strong ester bond and anticipated stability of this material, expressed the view that long-term and reproductive toxicity studies should be performed on the specific substance, as opposed to unmodified resin, before further evaluation. Because of early concerns on the part of the Committee about the lack of food-grade specifications for glycerol ester of wood rosin, plans for a further toxicological evaluation had to be postponed until such specifications were adopted at the thirty-seventh meeting of the Committee (Annex 1, reference *94*). The specifications

define the material as a complex mixture of tri- and diglycerol esters of resin acids from wood rosin.

Several recent studies, including a metabolic study in rats, a 13-week toxicity study in rats and mutagenicity studies, have been conducted on glycerol ester of wood rosin. In the 13-week toxicity study, the NOEL was 2500 mg per kg of body weight per day, the highest dose tested. Mutagenicity studies were negative.

The results of the recent metabolic study showed that glycerol ester of wood rosin given to rats in the diet was, for the most part, recovered unchanged in the faeces, and suggested that it was not hydrolysed in the gut to a significant extent and was largely unabsorbed. However, the lack of sensitivity of the analytical method used was such that a firm conclusion could not be reached as to the non-bioavailability of glycerol ester of wood rosin and/or its component resin acids.

Absorption studies with tritiated resin acids from wood rosin (e.g. dehydroabietic, tetrahydroabietic and isopimaric acids) indicated that more than 90% were recovered in urine or faeces within 2 weeks (most within 4 days) after oral administration. The small amount of dehydroabietic acid absorbed appeared to have been metabolized in the liver to three or four uncharacterized metabolites, which were then excreted in the bile and urine. Little evidence was found to show that tetrahydroabietic and isopimaric acids were metabolized.

The Committee noted the absence of adequate long-term toxicity/carcinogenicity studies and reproductive toxicity studies on glycerol ester of wood rosin. Because of the limited toxicological information available, the Committee was unable to establish an ADI. It considered that, as a minimum, studies demonstrating the metabolic stability and non-bioavailability of glycerol ester of wood rosin under conditions resembling those present in the human gastrointestinal tract would be required to permit further evaluation of this material.

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

Sucrose esters of fatty acids and sucroglycerides

Sucrose esters of fatty acids and sucroglycerides were previously considered by the Committee at its thirteenth, seventeenth, twentieth, twenty-fourth, thirty-fifth and thirty-ninth meetings (Annex 1, references *19, 32, 41, 53, 88* and *101*). At the thirty-ninth meeting, the Committee established a group ADI of 0–16 mg per kg of body weight for sucrose esters contained in sucrose esters of fatty acids and sucroglycerides, based on a NOEL for decreased body-weight gain of 50 g/kg in the diet, equal to 1.6 g per kg of body weight per day, observed in a long-term carcinogenicity study in rats with a palm-oil sucroglyceride. The NOEL was corrected for the content of mono- and diglycerides in the test material since these substances were considered to be normal constituents of the human diet. Because sucrose esters are hydrolysed in

the gut to normal dietary constituents prior to absorption, the Committee used a safety factor of 50 in deriving the ADI.

Since the last evaluation, new long-term toxicity/carcinogenicity and pharmacokinetic studies have become available in which the test material was composed entirely of sucrose esters of stearic and palmitic acids (70:30), with traces of oleic acid esters. In contrast to the sucrose esters previously tested, which consisted mainly of mono- and diesters, together with traces of triesters, the test material administered to rats in these studies contained 28% monoesters, 34% diesters, 21% triesters and 10% higher esters, while that administered to humans and dogs contained 57% monoesters, 28% diesters, 10% triesters and 1% higher esters. Neither of these sucrose ester formulations contained any mono- or diglycerides.

No adverse effects of treatment were demonstrated in the long-term toxicity/carcinogenicity study conducted in rats at dose levels of up to 50 g/kg in the diet, equal to 1970 mg per kg of body weight per day.

A series of studies on the disposition of two of these products in rats, dogs and humans demonstrated that small amounts of the monoesters were absorbed in all three species. On the basis of tissue residue and excretion studies in rats, it appears unlikely that diesters were absorbed. The small amounts of monoesters absorbed intact were completely metabolized and either excreted as carbon dioxide or integrated into body components. In humans, 20–30% of a dose of sucrose esters was retrieved intact from the faeces following a single or multiple dosing regimen, suggesting that 70–80% of the dose was hydrolysed to sucrose and component fatty acids.

The results of a tolerance study in humans conducted with sucrose esters administered in orange juice or with bread were also available. Although there were significant deficiencies in this study, most notably in the size of the dose groups and the lack of any controls, the results, indicating laxation and related abdominal symptoms with treatment at single doses of 1.5–3.0 g or divided doses of 3.0–4.5 g per day for 5–7 days, were of concern. A divided dose of 2.0 g per day for 5 days, equal to 35 mg per kg of body weight per day, produced no effect when administered in orange juice, and only a slight effect in 1/5 subjects when administered with bread. Only the highest dose tested, namely 4.5 g per day for 7 days, equal to 70 mg per kg of body weight per day, resulted in multiple occurrences of gastrointestinal symptoms (soft stools, diarrhoea, flatulence, borborygmus and bloated sensation) in the subjects. The results in humans were of interest since doses of 2000 mg per kg of body weight per day did not induce gastrointestinal disturbances in rats.

In view of the reservations about the tolerance study in humans, and a demonstrated NOEL in the rat of approximately 2000 mg per kg of body weight per day for sucrose esters of fatty acids, the Committee allocated a temporary group ADI of 0–20 mg per kg of body weight for the sucrose ester content of sucrose esters of fatty acids and sucroglycerides, and

requested the results of a well designed and conducted tolerance study in humans for review in 1997.

The Committee stressed that this evaluation applied to mono-, di- and tri-esters of sucrose with palmitic, stearic and oleic acids and to the sucrose ester content of sucroglycerides. It also stressed that the toxicological evaluation applied only to sucrose esters of fatty acids and sucroglycerides as currently specified, and not to materials characterized by higher levels of esterification.

An addendum to the toxicological monograph was prepared. The existing specifications for sucrose esters of fatty acids were revised. The specifications for the sucrose ester content of sucroglycerides were not reviewed.

3.1.5 *Flavouring agent*

Ethyl vanillin

Ethyl vanillin was first evaluated at the eleventh meeting of the Committee (Annex 1, reference *14*), when an ADI of 0–10 mg per kg of body weight was allocated on the basis of a long-term study in rats. At that time, the Committee noted that few metabolism studies had been carried out on ethyl vanillin and concluded that further studies of that type were desirable. Ethyl vanillin was re-evaluated at the thirty-fifth meeting of the Committee (Annex 1, reference *88*) on the basis of the partial application of the procedure for setting priorities for the safety review of food flavouring ingredients (Annex 1, reference *83*). At that time, the Committee noted that none of the previously evaluated long-term toxicity or carcinogenicity studies met modern standards in that fewer animals per group had been used than would be the present norm, and it therefore reduced the ADI to 0–5 mg per kg of body weight and made it temporary. The Committee requested submission of the results of adequate short-term toxicity and metabolism studies in rats for evaluation in 1992. At the thirty-ninth meeting (Annex 1, reference *101*), the Committee was informed that the studies requested had been initiated, and that preliminary results did not indicate any cause for concern. On the basis of this information, the Committee extended the previously allocated temporary ADI of 0–5 mg per kg of body weight, pending the submission of the final results of the ongoing short-term toxicity and metabolism studies in rats for evaluation by 1994.

At the present meeting, the Committee reviewed the studies requested. The metabolism studies indicated that ethyl vanillin was rapidly absorbed, metabolized and excreted in the rat. The principal metabolite identified was ethyl vanillic acid (3-ethoxy-4-hydroxybenzoic acid). This compound, which is not a normal constituent of human urine, has also been found in the urine of humans known to have ingested vanilla-flavoured foodstuffs.

In the recent 13-week toxicity study in which rats were fed ethyl vanillin at 500, 1000 or 2000 mg per kg of body weight per day, treated males

showed a transient reduction in body-weight gain compared with controls during the first 4 weeks of treatment. Since this effect was only transient and associated with reduced food intake, probably due to impaired palatability, the Committee concluded that the NOEL was 500 mg per kg of body weight per day.

The Committee considered ethyl vanillin not to be genotoxic on the basis of negative results in a large number of studies, although one assay for sister chromatid exchange was positive.

The Committee concluded that, in the light of the information showing daily intakes to be in the range 0.06–7 mg per person per day, the safety evaluation could be based on the principles applicable to materials occurring in foods in small amounts. In view of the limited toxicological information available, the Committee withdrew the previous temporary ADI and allocated an ADI of 0–3 mg per kg of body weight for ethyl vanillin, based on a NOEL of 500 mg per kg of body weight per day in the 13-week toxicity study in rats and a safety factor of 200.

A toxicological monograph, including relevant information from the previous monograph and information received since the previous evaluation, was prepared. The existing specifications were maintained.

3.1.6 *Food colours*

Canthaxanthin

Canthaxanthin was previously evaluated at the tenth, eighteenth, thirty-first and thirty-fifth meetings of the Committee (Annex 1, references *13, 35, 77* and *88*). At the thirty-first meeting, the Committee noted that canthaxanthin had been used as a direct food additive, as a feed additive, and as an orally administered pigmenting agent for human skin in both pharmaceutical and cosmetic applications. The previous ADI was reduced to 0–0.05 mg per kg of body weight and made temporary pending submission of:

(a) details of ongoing long-term studies in rats and mice;
(b) clarification of the factors that influence pigment deposition in the eye, including the establishment of the threshold dose, the influence of dose and duration of exposure, the reversibility of pigment accumulation, and the investigation of potential animal models; and
(c) clarification of whether pigment deposition is causally related to impaired ocular function.

At the thirty-fifth meeting, the Committee concluded that the long-term toxicity of canthaxanthin in rats indicated potential hepatotoxicity in humans. However, it considered that the main problem associated with canthaxanthin was the deposition of crystals in the human retina. In view of the irreversibility or very slow reversibility of such retinal crystal deposition, the significance of which was not known, the Committee was unable to establish an ADI for canthaxanthin when used as a food

additive or animal feed additive. The previous temporary ADI was therefore not extended.

Since the last review, several studies have been conducted in order to identify a suitable animal model for the deposition of canthaxanthin crystals in the retina. In cynomolgus monkeys, feeding with canthaxanthin for 2.5 years resulted in a dose-dependent accumulation of this substance in the retina. Although not visible by conventional ophthalmoscopy, birefringent inclusions were observed microscopically in the inner retinal layers, with a distribution similar to that seen in human canthaxanthin retinopathy. The NOEL in this study was 0.2 mg per kg of body weight per day.

A dose-response relationship between canthaxanthin intake and the development of crystalline deposits in the retina of humans had not previously been definitely established. However, a comprehensive retrospective biostatistical study of both unpublished and published studies, which included data on total intake ranging from 0.6 to 201 g over a period of 1-14 years, showed a strong dose-response relationship, suggesting a NOEL for canthaxanthin crystalline deposits in the human retina below a daily intake of 30 mg of canthaxanthin per person.

In 27 human subjects, some of whom received canthaxanthin for the first time while others had been treated for up to 10 years, no impairment of vision, as measured by electroretinography as a reduction in the scotopic B-wave amplitude, was observed at a daily intake of 15 mg of canthaxanthin per person (equivalent to 0.25 mg per kg of body weight per day) over a period of 5 weeks. An additional month on a dosage of 60 mg per person per day produced a reduction in scotopic B-wave amplitude, which was more pronounced after a further month of treatment with 90 mg of canthaxanthin per person per day.

Additional long-term toxicity/carcinogenicity studies in rats confirmed that canthaxanthin, as previously observed, was hepatotoxic in this species, but provided no evidence of carcinogenicity. At low doses (5 or 25 mg per kg of body weight per day) only sporadic occurrence of vacuolated liver cells was observed, and at higher dose levels (75 or 250 mg per kg of body weight per day) this change appeared reversible. The NOELs were 5 and 25 mg per kg of body weight per day in female and male rats, respectively. In contrast to the liver cell changes observed in rats, no such changes were seen in monkeys given up to 49 mg of canthaxanthin per kg of body weight per day for up to 2.5 years.

Hepatotoxicity in humans due to ingestion of canthaxanthin has not been reported and, although the number of cases was limited, no signs of hepatotoxicity were seen in patients with erythropoietic protoporphyria treated with a total of 3-150 g of canthaxanthin over a period of 1-12 years.

The Committee allocated an ADI of 0-0.03 mg per kg of body weight to canthaxanthin, based on a NOEL of 0.25 mg per kg of body weight per day in humans and a safety factor of 10.

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

Curcumin

Turmeric and curcumin (the main colouring component of turmeric) were considered at the thirteenth, eighteenth, twenty-second, twenty-fourth, twenty-sixth, thirtieth and thirty-fifth meetings of the Committee (Annex 1, references *19, 35, 47, 53, 59, 73* and *88*).

At the eighteenth meeting, a temporary ADI of 0–0.1 mg per kg of body weight was established for curcumin based on the ADI for turmeric and an assumed average level of 3% curcumin in turmeric (Annex 1, reference *35*). The temporary ADI for curcumin was extended after the twenty-second, twenty-fourth, twenty-sixth and thirtieth meetings, following the evaluation of new data (Annex 1, references *47, 53, 59* and *73*).

The temporary ADI of 0–0.1 mg per kg of body weight for curcumin was again extended at the thirty-fifth and thirty-ninth meetings (Annex 1, references *88* and *101*). At the latter meeting, the Committee requested the results of carcinogenicity studies in mice and rats given turmeric oleoresin (which were known to have been completed) and the results of a reproductive toxicity/teratogenicity study with curcumin.

The results of the carcinogenicity studies, together with new biochemical and genotoxicity data, were available to the Committee for evaluation. Information previously requested on the reproductive effects of curcumin was not provided, although a published reproductive toxicity study on turmeric was available.

In a study previously evaluated by the Committee, extracts of turmeric reportedly affected reproduction in rats when administered by gavage on days 1–7 of gestation at doses of 100 or 200 mg per kg of body weight per day. However, no reproductive effects were reported in Wistar rats fed 500 mg per kg of body weight per day of turmeric or 60 mg per kg of body weight per day of an alcoholic extract of turmeric (providing an equivalent dose of curcumin) in a multigeneration reproductive toxicity study. Data from multigeneration reproductive and/or teratogenicity studies with curcumin itself (rather than turmeric) were not available.

Of a single oral dose of 400 mg [^{3}H]curcumin (equivalent to 2000 mg per kg of body weight) administered to rats, only 60% of the radioactivity was excreted by 12 days. At lower doses (equivalent to 50 or 400 mg per kg of body weight), most of the dose was excreted within 72 hours.

No genotoxicity studies with high-purity curcumin were available. In limited studies with curcumin preparations of up to 85% purity, or of unknown purity, no mutagenic activity was seen in bacteria and only equivocal activity in assays for the induction of chromosomal aberrations. The Committee concluded that there was no evidence to show that curcumin was genotoxic.

Long-term studies on the carcinogenic potential of turmeric oleoresin containing a high percentage of curcumin (79–85%) have been completed in mice and rats at dose levels of 2000, 10 000 or 50 000 mg/kg in the diet, equal to 220, 1520 or 6000 mg per kg of body weight per day in mice and 80, 440 or 2000 mg per kg of body weight per day in rats. The authors noted statistically significant increases in the incidences of hepatocellular adenomas (mid-dose males and females), small intestinal carcinomas (low- and mid-dose males) and pituitary gland adenomas (highest-dose females) in mice and clitoral gland adenomas (females) in rats. On the basis of the results of these studies, the Committee concluded that the effects were not dose-related, and that curcumin was not a carcinogen.

Gastrointestinal irritation (ulcers, hyperplasia and inflammation) was common in male and female rats in the highest-dose groups, but was not observed in mice. The NOEL for gastrointestinal effects in rats was 10 000 mg/kg in the diet, equal to 440 mg per kg of body weight per day.

After 15 months of treatment, absolute and relative liver weights were increased in both male and female mice in the mid- and highest-dose groups relative to controls. The NOEL for liver enlargement was 2000 mg/kg in the diet, equal to 220 mg per kg of body weight per day.

On the basis of the NOEL of 220 mg per kg of body weight per day in the carcinogenicity study in mice and a safety factor of 200, the Committee increased the temporary ADI to 0–1 mg per kg of body weight and extended it, pending the submission of the results of a reproductive toxicity study with curcumin for review in 1998. The Committee has made repeated requests since the eighteenth meeting in 1974 for such a study. At its present meeting, it reconfirmed the need for the study and reiterated the view that previous studies with turmeric were not relevant to the evaluation of curcumin. If such studies are not submitted for review in 1998 it is unlikely that the temporary ADI can be further extended.

An addendum to the toxicological monograph was prepared. The existing specifications were maintained.

3.1.7 *Glazing agents*

Mineral oils and waxes

Food-grade mineral oils were last evaluated by the Committee at its thirty-seventh meeting (Annex 1, reference *94*). At that meeting, it reconsidered two 90-day feeding studies in Fischer 344 (F344) rats given 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, deposition in the liver, spleen and lymph nodes was again reported, but no haematological investigations were conducted. The Committee considered that both effects required further investigation and recommended that an adequate long-term feeding study should be performed using food-grade mineral

oils representative of those in commercial use. The temporary ADI "not specified" was extended.

Petroleum jelly was last evaluated at the thirty-third meeting (Annex 1, reference *83*). At that meeting, the Committee reiterated its concern about whether the chemical composition of petroleum jelly in current use met the specifications for the materials tested in toxicological studies submitted to it. It concluded that, for newer formulations of petroleum jelly, new specifications were required and that adequate long-term toxicity, mutagenicity, and reproductive toxicity/teratogenicity studies should be completed. No ADI was allocated.

Paraffin wax and microcrystalline wax were last evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference *101*). At that meeting, the Committee prepared separate specifications for these waxes and concluded that, because these specifications limit the number of waxes that can be used for food applications as compared with those tested in previous studies, previous long-term toxicity studies were suitable for evaluating the safety of hydrocarbon waxes in current use. The Committee reviewed the results of extraction and migration tests on waxes and wax-bearing products, information on the absorption and metabolism of hydrocarbon waxes, a long-term feeding study in Sprague–Dawley rats and a series of 180-day feeding studies in rats, and concluded that petroleum-derived paraffin and microcrystalline waxes were non-toxic and non-carcinogenic. A group ADI "not specified" was established for microcrystalline wax and paraffin wax for the uses indicated in the specifications (chewing-gum base, protective coating, defoaming agent and surface-finishing agent). The Committee was informed that a 90-day study on hydrocarbon waxes made both by newer processes and by traditional methods was under way, and asked to be informed of the results when they became available.

At the present meeting, the Committee reviewed the results of the above study, together with the results of three other recent 90-day studies in F344 rats on a range of mineral oils and waxes representative of materials currently in use. The materials tested were as follows:

- *Mineral oils*:[1] N10(A), N15(H), P15(H), N70(A), N70(H), P70(H) and P100(H).
- *Paraffin waxes:* low-melting-point wax (LMPW) and intermediate-melting-point wax (IMPW).
- *Microcrystalline waxes:* high-melting-point wax (HMPW) and high-sulfur wax (HSW).

[1] Oils may be obtained from crude oil sources of naphthenic (N) or paraffinic (P) origin and by either the conventional acid (oleum)-treatment process (A) or the hydrogenation or hydrotreatment process (H). Their viscosity ranges from 10 to 100 centistokes (cSt) (10–100 mm^2/s). Thus a P100(H) oil refers to a paraffinic oil with a viscosity of 100 cSt produced by the hydrogenation process and a N10(A) oil to a naphthenic oil with a viscosity of 10 cSt produced by the acid-treatment process.

The dose levels used were 0.002%, 0.02%, 0.2% or 2% in the diet (equivalent to 2, 20, 200 or 2000 mg per kg of body weight per day), except for IMPW, for which the lowest dose level was 0.02% (equivalent to 20 mg per kg of body weight per day).

In these studies, neither HSW nor HMPW accumulated in any tissues or produced any effects. The P100(H) oil produced no effects but did accumulate in the liver to a small extent at the highest dose level. The P70(H) oil accumulated in the liver, kidney and mesenteric lymph nodes when administered at the highest dose level, but the only treatment-related effect, which was seen at this level only, was described in the study report as an increased incidence of pigmented macrophages in the lymph nodes. With all the other materials, there was evidence of accumulation of test material and effects indicative of a reaction to a foreign body at one or more dose levels. The types of effects seen were similar in nature and included focal histiocytosis; increases in the weight of the liver, lymph nodes, spleen and kidney; granulomas or micro-granulomas in the liver; haematological changes typical of a mild, chronic inflammatory reaction; and biochemical changes indicative of mild hepatic damage. These effects were similar to those seen in the two 90-day studies with mineral oil considered by the Committee at its thirty-seventh meeting.

The incidence of inflammatory lesions in the mitral valve of the heart was significantly increased in rats fed 0.2% or 2% LMPW and occasionally in rats fed other materials. Such lesions were also seen occasionally in control rats. The Committee considered that the significance and treatment-related incidence of these lesions could be clarified by a re-examination of the histological data on all treated and concurrent control groups in the recent studies and in historical controls.

The Committee considered that, although the types of effects seen were essentially reactions to a foreign body, it was possible that a prolonged inflammatory response of this type could result in functional changes in the immune system and that this aspect required further investigation.

The NOELs in these studies are given in Table 1. Except for the P70(H) oil and those materials showing no effects, the NOELs were based on an increased incidence of histiocytosis in the lymph nodes at the next highest dose level. For the P70(H) oil, the NOEL was based on an increased incidence of pigmented macrophages in the lymph nodes at the 2% dose level.

Two of the studies included a reversal period of either 28 or approximately 90 days, but most of the toxicological effects were still evident at the end of this period. The Committee noted that there was limited evidence that the severity of some of these effects had decreased during this phase, although it appeared that a period longer than 90 days would be required in order to determine whether the effects produced by these materials are fully reversible.

Table 1
NOELs and ADIs for mineral oils, paraffin waxes and microcrystalline waxes tested in 90-day studies in F344 rats

Substance	NOEL (mg per kg of body weight per day)	ADI (mg per kg of body weight)
LMPW	< 2	ADI withdrawn[a]
IMPW	< 2	ADI withdrawn[a]
N10(A) oil	2	0-0.01[b]
N15(H) oil	< 2	0-0.01[b]
P15(H) oil	2	0-0.01[b]
N70(A) oil	2	0-0.01[b]
N70(H) oil	2	0-0.01[b]
P70(H) oil	200	0-1[c]
P100(H) oil	2000	0-20
HSW	2000	0-20[d]
HMPW	2000	0-20[d]

[a] Previous ADI "not specified".
[b] Temporary group ADI.
[c] Temporary ADI.
[d] Group ADI.

The Committee recalled that much earlier rat studies on mineral oils and waxes, of duration ranging from 90 days to lifetime and utilizing strains of rat other than F344, had shown no adverse toxicological effects. Although many of these studies had design and/or reporting deficiencies, the lack of significant findings in other strains suggested to the Committee that the F344 rat may be especially sensitive. In one of the studies considered at the meeting, the responses of F344 and Sprague-Dawley rats to P15(H) oil were compared, and it was found that the latter were less susceptible to the toxic effects of the test materials, although absorption and organ weight and histopathological changes were evident in both strains.

The Committee also noted the limited data available on mineral hydrocarbons from human studies, which showed that mineral-oil-induced lesions similar to those seen in rats have been identified in human tissues. Vacuoles of accumulated mineral oil have been found in the liver, spleen and lymph nodes. Some studies reported the presence of an accompanying inflammatory or granulomatous reaction, while others reported no tissue reaction to the accumulated material. However, none of the studies contained detailed information about the individuals' history

of use of liquid paraffin as a medicine nor their dietary intake. It was therefore not possible to assess the level of intake of mineral oil associated with hydrocarbon accumulation in humans.

Food-grade mineral oils and paraffin and microcrystalline waxes are complex mixtures of hydrocarbons and other materials. It was evident from the results of the new 90-day studies that the absorption and subsequent toxicity of these materials are associated with their physical properties rather than the crude oil source or refining method. The oils and waxes with a high NOEL contain a greater proportion of hydrocarbon components of high relative molecular mass (high carbon number) and have higher viscosities than those with a low NOEL, which contain a greater proportion of hydrocarbon components of lower relative molecular mass (low carbon number). Mineral hydrocarbons are specified, on the whole, by means of ranges of physical and chemical parameters. The results of the studies considered at the present meeting were therefore applicable not only to the particular materials tested but also to other mineral hydrocarbons having physical and chemical parameters falling within the same ranges.

The Committee decided to withdraw, alter or allocate ADIs for mineral oils and waxes as set out in Table 1.

For paraffin waxes, the previous ADI "not specified" was withdrawn because toxicological effects were observed at all dose levels.

A group temporary ADI of 0-0.01 mg per kg of body weight was allocated to mineral oils falling within the specifications for the N10(A), N15(H), P15(H), N70(A) and N70(H) oils and a temporary ADI of 0-1 mg per kg of body weight was allocated to mineral oils meeting the specifications for the P70(H) oil. The Committee requires information about the compositional factors in mineral oils that influence their absorption and toxicology for review in 1998. It also requires a study of at least 1 year's duration on one of these materials in F344 rats, which should include an assessment of immune function at appropriate time periods and an investigation of the kinetics of accumulation of the material, and particularly whether a plateau is reached. A reversal period of 1 year should also be included, in order to determine whether the granulomatous hepatic lesions observed in rats in the 90-day studies are fully reversible. The results of this study, together with all relevant background data, including the physical and chemical parameters of the materials tested, should be submitted for review in 1998.

For those materials that caused no adverse effects at the highest level tested, the Committee concluded that, although the NOEL was derived from a study of only 90 days' duration, the dose administered was sufficiently high to provide reassurance that accumulation of these substances was unlikely to occur following longer-term administration. An ADI of 0-20 mg per kg of body weight could therefore be established for mineral oils meeting the specifications for the P100(H) oil and a

group ADI[1] of 0–20 mg per kg of body weight could be established for waxes meeting the specifications for HSW and HMPW.

Insufficient information was available for the Committee to re-evaluate petroleum jelly. This substance should be considered at a future meeting.

An addendum to the toxicological monograph for mineral oils, microcrystalline wax and paraffin wax was prepared.

The existing specifications for microcrystalline wax were revised to cover the waxes for which an ADI of 0–20 mg per kg of body weight has been set. As the Committee decided to withdraw the ADI for other waxes, the existing specifications for paraffin wax were withdrawn.

The existing specifications for mineral oil were revised and, in order to define more clearly the materials evaluated by the Committee, these products were divided into two groups. Specifications for mineral oil (high-viscosity) and mineral oil (medium- and low-viscosity) were therefore prepared. The specifications for mineral oil (high-viscosity) cover the oil for which an ADI of 0–20 mg per kg of body weight has been allocated. The specifications for mineral oil (medium- and low-viscosity) cover the products for which temporary ADIs have been allocated. These specifications define three classes of oils, namely class I, including the medium-viscosity oil P70(H) (temporary ADI of 0–1 mg per kg of body weight), class II, including the medium-viscosity oils N70(H) and N70(A) (temporary ADI of 0–0.01 mg per kg of body weight), and class III, including the low-viscosity oils P15(H), N15(H) and N10(A) (temporary group ADI of 0–0.01 mg per kg of body weight).

The existing tentative specifications for petroleum jelly were revised and the "tentative" designation was maintained.

3.1.8 *Sweetening agent*

Alitame

Alitame has not been previously evaluated by the Committee. A number of toxicity studies on this substance were available, as well as studies on the β-isomer of alitame, which is formed at low levels in some foods.

In metabolism studies conducted in three animal species and in humans, alitame was readily absorbed from the gastrointestinal tract and then rapidly metabolized and excreted. In mice, rats and dogs, the route of metabolism involved cleavage of the peptide bond yielding aspartic acid and D-alanine tetramethylthietane amide, the latter being further biotransformed by oxidation at the thietane sulfur to form the sulfoxide and sulfone. Acetylated derivatives of these metabolites were commonly found in the urine of rats and dogs, whereas, in humans, the glucuronide derivative of D-alanine tetramethylthietane amide was the major urinary

[1] A group ADI was established since both waxes meet the same specifications.

metabolite. In rats, initial cleavage of the peptide bond of alitame took place in the lumen of the jejunum. In pregnant rats, alitame was readily transferred transplacentally, but there was no evidence of active secretion in the milk. In a study in rats, levels of alitame residues decreased rapidly in all tissues except the eyes. Further studies indicated that it was bound to melanin contained in the uveal tract (choroid, ciliary body and iris) and suggested that such binding was associated with a metabolite formed only in rats. The Committee considered that an *in vivo* study in a mammalian species other than the rat is desirable to clarify the significance of the retention of alitame in the eyes.

The β-isomer of alitame was rapidly metabolized in rats, the only urinary metabolite not also formed with alitame being the *N*-acetyl derivative of this isomer. There was no evidence of the formation of significant amounts of the β-isomer of alitame in the stomach or small intestine of the rat.

Acute and short-term toxicity studies indicated that the toxicity of alitame is low. In studies conducted in mice, rats and dogs, the major toxicological changes observed were associated with the liver. A dose-related increase in liver weight and evidence of centrilobular hypertrophy were seen in all of the studies in these three species, and these changes were accompanied by an increase in the level of hepatic microsomal enzymes. Short-term studies on hepatic enzyme induction indicated that alitame was a weak inducer of microsomal enzymes; the pattern of induction was qualitatively similar to that caused by phenobarbitone but at a lower level. The liver changes were indicative of an adaptive response and, in rats and dogs, withdrawal of treatment after a 3-month period resulted in partial or complete reversal of the observed changes within a month.

Two-year carcinogenicity studies have been conducted in CD-1 mice, Long-Evans rats and Sprague-Dawley rats. The two studies conducted in rats included an *in utero* phase. There was no evidence of carcinogenicity in the study in mice. In rats, the first carcinogenicity study was conducted in Long-Evans rats fed diets containing 0.1%, 0.3% or 1% alitame and, while the survival rate was greater than 50% in all groups at 22 months, poor survival was noted at 24 months. Pathological examination of the liver indicated a higher incidence of focal nodular hyperplasia and eosinophilic foci in females in the highest-dose group than in controls, according to the diagnostic criteria of Squire & Levitt (*6*). Two subsequent independent re-examinations of these data have been performed using the diagnostic criteria of Maronpot et al. (*7*). In the first re-examination (1987), the higher incidence of eosinophilic foci in females in the highest-dose group was confirmed, but no increase was seen in the incidence of focal hyperplasia or hepatocellular adenomas. In the second re-examination (1994), the higher incidence of eosinophilic foci in females in the highest-dose group was again confirmed. A higher incidence of these foci was also found at the mid-dose level. Also in this re-examination, the nodular hyperplastic lesions noted in the original

report were largely reclassified as hepatocellular adenomas, and an increased incidence of these lesions was seen at the highest dose level. No evidence of hepatocellular carcinomas was found in female rats in the original examination or in the subsequent re-examinations. The main difference between the first and second re-examinations was in the nomenclature used, rather than in the recognition that lesions existed. The Committee expressed concern at the change in the way that these liver lesions were classified, possibly as a result of the use of different diagnostic criteria. It considered that there was evidence of a higher incidence of adenomas in treated females than in controls in this study, at least at the highest dose level. The adenomas were found late in the study and, while considered unlikely to progress to hepatocellular carcinomas, the data were inconclusive in this regard.

A second carcinogenicity study, this time in Sprague-Dawley rats, was conducted because of the low survival rate at 24 months in the Long-Evans rats. In this second study, the survival rate was lower at both 22 and 24 months than that found in the Long-Evans rats, and hence the study was considered inadequate to provide further information regarding the potential carcinogenicity of alitame.

The Committee considered that the available studies did not indicate that alitame was carcinogenic, but that they were deficient in certain respects and did not fully address this question. It also considered that further research to clarify the mechanism of the formation of adenomas in female Long-Evans rats was desirable.

Genotoxicity studies both *in vitro* and *in vivo* did not provide any evidence that alitame has a mutagenic potential. Similarly, there was no evidence from studies conducted in rats and rabbits that alitame has a teratogenic potential.

Two reproductive toxicity studies (2-generation) were conducted in Long-Evans rats and one reproductive toxicity study (1-generation) in Sprague-Dawley rats. The first reproductive toxicity study with Long-Evans rats produced some evidence for slightly decreased body-weight gain in the pups during lactation at 1% in the diet, the highest dose administered. A similar effect was noted in the second reproductive toxicity study at the same dose level, but not in the 1-generation reproductive toxicity study with Sprague-Dawley rats. A study of the effects of spilt feed containing alitame in the bedding material, while providing a possible explanation for the decrease in pup body-weight gain, did not fully address this issue. A slight effect of alitame on locomotor activity seen in the first reproductive toxicity study was not substantiated in subsequent studies. The Committee did not regard the changes seen in the reproductive toxicity studies to be of toxicological significance.

Neurotoxicity and neurobehavioural toxicity studies in rats using a battery of observational tests indicated behavioural changes only when

very high doses were administered as a single dose by gavage (5000 mg per kg of body weight). These behavioural changes were of short duration and animals appeared to be normal by day 7. There was no evidence of neurotoxicity in rats fed diets containing 1% alitame (equivalent to 1000 mg per kg of body weight per day) over a 3-month period. Similarly, no changes indicative of neurotoxicity were observed in dams or pups when the same dose level was given over the reproductive period.

In a 14-day study conducted in humans there was no evidence of hepatic enzyme induction at a dose level of 15 mg per kg of body weight per day.

Ninety-day tolerance studies were conducted in normal and diabetic subjects. There was no evidence of adverse effects in subjects of both types during the period of the study at a dose level of 10 mg per kg of body weight per day. However, in the 1- and 2-year follow-up of the diabetic subjects, there was a higher incidence of myocardial infarction in the treatment group than in the controls, namely 5/58 in the treatment group and 0/71 in the controls after 1 year, and 6/53 in the treatment group and 3/67 in the controls after 2 years. While the differences at 2 years were not statistically significant, and there was no indication of cardiovascular effects in the animal studies, the Committee considered that the increased incidence of myocardial infarction in the treatment group has not been fully explained. It was advised that a further study is to be performed in diabetic subjects.

Studies conducted with the β-isomer of alitame provided no evidence of genotoxicity, teratogenicity or systemic toxicity in mice or rats at dose levels of up to 25 mg per kg of body weight per day.

The Committee recognized that the basis on which to consider establishing an ADI for a chemical such as alitame is difficult to specify, since the most sensitive treatment-related effects observed were liver enzyme changes and liver weight changes, which may be adaptive in nature. For alitame, these changes appeared to be closely linked. While the prolonged hepatomegaly observed was not accompanied in the long-term studies by any pathological changes indicative of toxicity, it is not considered desirable. On the other hand, moderate induction of hepatic enzymes is considered a normal adaptive process. In the case of alitame, significant changes in body-weight gain or liver weight were observed at doses above the NOELs. In the 18-month study in dogs, the NOEL was 100 mg per kg of body weight per day. In the lifetime studies in rats, the NOEL was 0.3% in the diet, equal to 130 mg per kg of body weight per day in Long-Evans rats and 230 mg per kg of body weight per day in Sprague-Dawley rats.

The Committee concluded that the concerns raised by the deficiencies in the carcinogenicity studies in rats were of such significance that an ADI could not be allocated.

A toxicological monograph and new specifications were prepared.

3.1.9 *Thickening agent*

Processed Eucheuma seaweed

Processed *Eucheuma* seaweed was previously considered by the Committee at its thirtieth, thirty-ninth and forty-first meetings (Annex 1, references *73, 101* and *107*). At the forty-first meeting, the Committee reviewed a 90-day study in rats and a series of genotoxicity studies on processed *Eucheuma* seaweed from *E. cottonii*. Since the histopathological data on individual animals in the 90-day study were not available at that time, the Committee established a temporary ADI of 0–20 mg per kg of body weight for processed *Eucheuma* seaweed from *E. cottonii*. These individual animal data were available at the present meeting and were considered together with new genotoxicity and cytotoxicity assays.

The Committee was informed that the carrageenan component of processed *Eucheuma* seaweed was essentially chemically identical to that of traditionally refined carrageenan obtained from the same sources. However, processed *Eucheuma* seaweed also contains a substantial amount of acid-insoluble material consisting mainly of cellulose. Because of the chemical relationship between processed *Eucheuma* seaweed and traditionally refined carrageenan, the Committee considered that toxicological data on the latter were relevant to the safety assessment of the carrageenan polysaccharide constituents of processed *Eucheuma* seaweed, but could not replace adequate toxicological studies on processed *Eucheuma* seaweed itself.

The individual animal data from the 90-day feeding study in rats confirmed the accuracy of the summary data and the conclusions derived from them. However, the Committee expressed reservations about the lack of documentation on good laboratory practices, the conduct of the histopathological examinations and the results themselves; in addition, the small numbers of animals tested were of concern. Following reassessment, none of the available genotoxicity studies were considered to be adequate because of deficiencies in their conduct or reporting.

In view of the above concerns about the available toxicity data on processed *Eucheuma* seaweed from *E. cottonii*, the Committee extended the temporary ADI of 0–20 mg per kg of body weight pending the submission of a new 90-day feeding study in rodents and an appropriate battery of genotoxicity studies, all meeting present-day standards, for processed *Eucheuma* seaweed derived from *E. cottonii*. These studies are required for review in 1998.

A request was made at the present meeting to amend the specifications to include *Eucheuma spinosum* as a source material for processed *Eucheuma* seaweed. However, no toxicological studies on material derived from *E. spinosum* were submitted.

In recognition of the fact that different effects have been noted in toxicity studies in the guinea-pig with ι-carrageenan, the major component of

E. spinosum, compared with κ-carrageenan, the major component of *E. cottonii* (Annex 1, reference *33*), a separate 90-day feeding study in rodents and a separate battery of genotoxicity studies are required for processed *Eucheuma* seaweed derived from *E. spinosum* if the specifications are to be expanded to include processed *Eucheuma* seaweed derived from this species. The Committee concluded that a complete review of all carrageenan data should be undertaken in 1998, particular attention being paid to the identity of the source materials and to the specifications of the products that have been tested.

In addition, the Committee was aware that the use of carrageenan as an adjuvant had raised concerns about its possible effects on the immune system.

The Committee was also informed that processed *Eucheuma* seaweed and traditionally refined carrageenans are being added in substantial amounts to foods subjected to cooking and frying. This raised the question of possible thermal degradation of the carrageenan polysaccharide to species of lower relative molecular mass which may be of toxicological significance.

An addendum to the toxicological monograph was not prepared. The existing specifications for processed *Eucheuma* seaweed from *E. cottonii* were revised.

3.2 Miscellaneous substances

3.2.1 *β-Cyclodextrin*

β-Cyclodextrin was previously evaluated at the forty-first meeting of the Committee (Annex 1, reference *107*), at which time a temporary ADI of 0-6 mg per kg of body weight was allocated, based on a NOEL of 2.5% in the diet (equal to 1230 mg per kg of body weight per day) in a study in dogs and a safety factor of 200. The results of an ongoing 1-year oral toxicity study in dogs and information on the effects of β-cyclodextrin on the bioavailability of lipophilic nutrients were required by 1995. These data, along with the results of a 1-year toxicity study in rats, a 3-generation reproductive toxicity study in rats and carcinogenicity studies in mice and rats were available for review at the present meeting.

The Committee considered that the further toxicity studies confirmed the low systemic toxicity of β-cyclodextrin. The NOEL in the 1-year studies in rats and dogs was 1.25% in the diet, equal to 650 and 470 mg per kg of body weight per day, respectively. At higher doses there were minor changes in blood biochemical and/or urinalysis parameters and, in the rat, minor necrotic and inflammatory changes in the liver and an increased incidence of pigment in cortical tubular epithelium in the kidney. The NOEL was also 1.25% in the diet (equal to between 560 and 2900 mg per kg of body weight per day, depending on the stage of the study) in the 3-generation reproductive toxicity study in rats, in which the only

adverse effect seen at higher doses was impaired pup growth during lactation, which was probably secondary to reduced food consumption and body-weight gain in the dams at this dose level.

In the carcinogenicity study in mice, β-cyclodextrin caused inflammatory changes in the lower gastrointestinal tract, which were considered to be the cause of death of some animals. The lowest dose level at which this occurred was 75 mg per kg of body weight per day (1/52 males affected) and the NOEL was 25 mg per kg of body weight per day. The Committee considered that these lesions probably represent a species-specific reaction to β-cyclodextrin in some mice. No such effects were seen either in the carcinogenicity study in rats, in which the concentration of β-cyclodextrin in the diet was about three times that in the diet of mice, or in shorter-term studies in rats and dogs at far higher β-cyclodextrin concentrations in the diet. No treatment-related neoplastic lesions were observed in the carcinogenicity studies.

An *in vitro* study indicated that β-cyclodextrin is unlikely to deplete lipophilic vitamins when used as an ingredient in food, since it has a higher affinity for bile acids than for vitamins A and D_3. The fact that laboratory animals experienced no apparent nutritional deficiencies affecting clinical status or survival following lifetime exposure to high levels of β-cyclodextrin in the diet also provides reassurance that the use of this additive will not adversely affect nutritional status in humans.

The Committee allocated an ADI of 0–5 mg per kg of body weight for β-cyclodextrin, based on the NOEL of 1.25% in the diet (equal to 470 mg per kg of body weight per day) in the 1-year study in dogs and a safety factor of 100.

An addendum to the toxicological monograph was prepared. The existing specifications were revised and the "tentative" designation was deleted.

3.2.2 *Nitrate and nitrite*

Intake

The natural occurrence of nitrates and nitrites in the environment is a consequence of the nitrogen cycle whereby nitrogen is fixed by bacteria as nitrate before use in the synthesis of plant proteins. Nitrate also occurs as a contaminant in drinking-water. Nitrate and nitrites are used as food additives, being added as preservatives and colour fixatives to some processed foods, particularly cured meats, where they control the growth of *Clostridium botulinum.* They are also used in the preservation of fish and in cheese production in some countries.

In this report, for the sake of consistency, levels of nitrate and nitrite are expressed as nitrate and nitrite ion.

Vegetables (including potatoes) constitute the major dietary source of nitrate, generally providing more than 85% of the daily dietary intake (*8*). Nitrate levels in vegetables vary widely (from 1 to 10 000 mg per kg),

depending on the type of vegetable, its source, and the conditions of cultivation and storage (*8, 9*). Leafy vegetables and some root crops (e.g. beetroot and radishes) frequently contain nitrate levels exceeding 2500 mg per kg (*10*). Reports of mean dietary intakes of nitrate in various countries range from 31 to 409 mg per person per day (*8, 11*), although not all surveys include drinking-water. Dietary intake of nitrate is significantly affected by cultural and lifestyle factors as well as by geographical location. Certain Asian populations, vegetarians and those exposed to high concentrations of nitrate in their drinking-water (> 50 mg/l) are more likely to have dietary intakes of nitrate above 220 mg per day. Drinking-water containing high levels of nitrate will also result in high intakes for infants fed formulations prepared with such water (e.g. 46 mg per day for water containing 50 mg/l) (*8*). In addition, endogenous synthesis of nitrate has been estimated to be about 1 mg per kg of body weight per day, and this may increase substantially in inflammatory conditions such as gastroenteritis.

Nitrite levels in most foods are very low (generally well below 10 mg per kg) and rarely exceed 100 mg per kg (*8*). Exceptions to this are vegetables that have been damaged or stored poorly or for extended periods and pickled or fermented vegetables. In such circumstances levels of up to 400 mg per kg have been found (*9*).

Nitrite present in cured meat has been reported to account for up to 70% of total dietary intake of this substance, depending on the intake of such meat and the origin and type of cured meat consumed (*12*). Reports of mean dietary nitrite intake from all food sources range from < 0.1 to 8.7 mg per person per day for European diets (*8*).

Oral reduction of the nitrate secreted in saliva also contributes to total exposure to nitrite. When conversion of nitrate to nitrite in the human body is taken into account, most of the nitrite to which populations are exposed comes from vegetables and less than 10% from cured meats (*13*).

Nitrosamines can be formed in food under suitable conditions as a consequence of chemical reactions between nitrosatable precursors in food (e.g. amines) and nitrosating agents (e.g. nitrite and nitrogen oxides).

The Committee emphasized the need for good manufacturing practice when nitrates and nitrites are used as food additives so as to ensure that the minimum amounts of these substances are used to achieve their functional purpose; nitrosation inhibitors (e.g. ascorbic acid) may also have to be used. Such practices will reduce the chances that nitrosamines will be formed.

The Committee noted several studies which showed that food preparation techniques such as malting, smoking, drying and broiling of meat and fish products, as well as frying of cured meats including bacon, can, under certain conditions, promote the formation of nitrosamines. It therefore emphasized the need for good manufacturing practices in the preparation of meat and fish products so as to reduce exposure to these compounds.

Nitrite (and potential endogenous formation of N-nitroso compounds)

Nitrite was reviewed at the sixth, eighth, seventeenth and twentieth meetings of the Committee (Annex 1, references *6, 8, 32* and *41*). At its sixth meeting, the Committee allocated an ADI of 0-0.4 mg per kg of body weight to this substance, expressed as sodium nitrite. This ADI was based on a marginal reduction in body-weight gain at a dose level of 100 mg per kg of body weight per day in a long-term study in rats. At its seventeenth meeting, the Committee lowered the ADI to 0-0.2 mg sodium nitrite per kg of body weight and made it temporary. At that time, the Committee used a safety factor higher than normal (500) because a marginal effect level was considered and there was a possibility of the endogenous formation of *N*-nitroso compounds from the nitrite and *N*-nitrosatable compounds present together in food and the gastrointestinal tract. At its twentieth meeting, the Committee considered the reports of a WHO task group (*14*) and of a working group of the International Agency for Research on Cancer on *N*-nitroso compounds (*15*), but concluded that they did not provide sufficient evidence to revise the temporary status of the ADI. Since the previous evaluation of nitrite, numerous toxicological and epidemiological data have become available.

The toxic effects of nitrite are of the following three types: (1) the formation of methaemoglobin; (2) hypertrophy of the adrenal zona glomerulosa in rats; and (3) genotoxicity.

Methaemoglobinaemia is seen particularly after acute and subacute exposure. However, it is not the sole determinant of the NOEL. In a 2-year oral toxicity study in rats, the NOEL was 6.7 mg nitrite per kg of body weight per day (67 mg/l of drinking-water per day), expressed as nitrite ion. At the next higher dose level of 67 mg nitrite per kg of body weight, methaemoglobin accounted for 5% of total haemoglobin; in addition, dilatation of coronary arteries and of the bronchi with infiltration of lymphocytes and alveolar hyperinflation were also seen. Methaemoglobin is particularly important where it exceeds 10% of total haemoglobin, leading to toxic effects such as cyanosis. Young infants (below the age of 3 months) seem especially vulnerable to methaemoglobin. There is also evidence that fetal haemoglobin is more readily oxidized to methaemoglobin, and that in the neonate methaemoglobin reductase is less effective in the reduction of methaemoglobin to normal haemoglobin.

In a 90-day toxicity study in Wistar rats, the incidence and degree of hypertrophy of the adrenal zona glomerulosa observed at a dose level of 5.4 mg per kg of body weight per day, expressed as nitrite ion, were not significantly different from those among controls, whereas at higher dose levels the hypertrophy was both significant and dose-related.

In another 90-day toxicity study carried out by other investigators with a different Wistar substrain, slight hypertrophy of the adrenal zona glomerulosa was seen from 28 days onwards, but only at dose levels three

times as high. The NOEL for hypertrophy in these studies was 5.4 mg per kg of body weight per day, expressed as nitrite ion.

Nitrite both with and without nitrosatable precursors was found to be genotoxic in several *in vitro* and *in vivo* test systems. However, DNA repair was not affected by nitrite.

Carcinogenicity studies with nitrite were negative, with the exception of those in which extremely high doses of both nitrite and nitrosatable precursors were administered. In addition, there was no evidence for an association between nitrite and nitrate exposure in humans and the risk of cancer. The Committee noted that few epidemiological studies were available in which cancers other than gastric cancer were investigated.

Although it has been shown in several controlled laboratory studies that, when both nitrite and *N*-nitrosatable compounds are present together at high levels, *N*-nitroso compounds are formed endogenously, there are quantitative data only on those *N*-nitroso compounds which are readily formed endogenously, such as *N*-nitrosoproline, which is not carcinogenic. As there was no quantitative evidence of the endogenous formation of carcinogenic *N*-nitroso compounds at intake levels of nitrite and nitrosatable precursors achievable in the diet, a quantitative risk assessment of nitrite on the basis of endogenously formed *N*-nitroso compounds was not considered to be appropriate. The safety evaluation was therefore based on the toxicity studies on nitrite.

As previously mentioned, the NOEL was 5.4 mg per kg of body weight per day (expressed as nitrite ion) in 90-day toxicity studies in rats in which hypertrophy of the adrenal zona glomerulosa was observed and 6.7 mg per kg of body weight per day (expressed as nitrite ion) in a 2-year toxicity study in rats in which toxic effects in the heart and lungs were observed. On the basis of these results and a safety factor of 100, the Committee allocated an ADI of 0–0.06 mg per kg of body weight to nitrite, expressed as nitrite ion. This ADI applies to all sources of intake. Nitrite should not be used as an additive in food for infants below the age of 3 months. The ADI does not apply to such infants.

A toxicological monograph summarizing both relevant information from the previous monograph and the information that has become available since the previous evaluation was prepared. The existing tentative specifications for potassium nitrite and sodium nitrite were revised, and the "tentative" designation was deleted.

Nitrate

This substance was considered at the sixth, eighth and seventeenth meetings of the Committee (Annex 1, references *6, 8* and *32*). At the sixth meeting, an ADI of 0–5 mg per kg of body weight, expressed as sodium nitrate, was allocated. This ADI was based on a NOEL for sodium nitrate of 500 mg per kg of body weight per day derived from a long-term toxicity study in rats and a short-term toxicity study in dogs together with

a safety factor of 100. Growth depression was observed at higher dose levels. The ADI of 0-5 mg per kg of body weight was retained at the eighth and seventeenth meetings.

Since the previous evaluation, new toxicological and epidemiological data have become available, which were reviewed at the present meeting. The Committee noted that nitrate *per se* can generally be considered to be of relatively low toxicity. However, it was aware that nitrite is formed in the human body by reduction of nitrate and that *N*-nitroso compounds can also be formed from nitrite and *N*-nitrosatable compounds under certain conditions. Thus, the assessment of the health risk of nitrate to humans should encompass the toxicity of both nitrite and *N*-nitroso compounds, and the animal species used for safety evaluation should be closely related to humans with respect to the toxicokinetics of nitrate and the conversion of nitrate to nitrite. Furthermore, in the toxicological evaluation of nitrate, it should be considered in conjunction with nitrite and potential endogenously formed *N*-nitroso compounds.

As the toxicity of nitrate results from its conversion to nitrite and the possible endogenous formation of *N*-nitroso compounds, and the toxicokinetics and biotransformation of nitrate in the rat are different from those in humans, rats are less suitable than rabbits, dogs and pigs for use in assessing the toxicity of nitrate in humans. However, the toxicological data are too limited to allow a safety evaluation on the basis of the results of studies on these species. For these reasons both the toxicity studies on nitrate in laboratory animals and those on nitrite in combination with data on the conversion of nitrate to nitrite were considered by the Committee.

The possible endogenous formation of *N*-nitroso compounds from nitrite and *N*-nitrosatable compounds as precursors has already been discussed (see p. 32). No evidence of an association between nitrate exposure and the risk of cancer was found in either the toxicological or epidemiological studies, and nitrate was not genotoxic.

In two long-term toxicity studies in rats, one old and one recent, doses of 370 and 1820 mg per kg of body weight per day, expressed as nitrate ion, respectively, failed to produce any effects. However, the second of these was solely a carcinogenicity study, in which the highest dose level of 1820 mg nitrate ion per kg of body weight per day could not be considered as a NOEL because complete histopathological examinations were not performed.

The experimental design of a recent study in rats on possible behavioural effects of nitrate was considered to be inappropriate for safety evaluation purposes.

A short-term toxicity experiment in pigs indicated that a daily dose level of 3% potassium nitrate, equivalent to 730 mg per kg of body weight per day expressed as nitrate ion, inhibited the functioning of the thyroid. This finding was supported by an epidemiological cohort study in which

enlargement of the thyroid and decreased levels of serum thyroid-stimulating hormone were seen at high nitrate levels in drinking-water.

In the light of the overall information on the toxicity of nitrate, the NOEL of 370 mg nitrate ion per kg of body weight per day was considered to be the most appropriate for safety evaluation.

If the proportion of nitrate converted to nitrite in humans is taken as 5% (mol/mol) for normally responding individuals and 20% (mol/mol) for those showing a high level of conversion and the NOEL for nitrite (6 mg per kg of body weight per day expressed as nitrite ion) is used, the NOELs for nitrate, expressed as nitrate ion, would be 160 and 40 mg per kg of body weight per day, respectively. As these figures are derived in part from human pharmacokinetic data, the use of a safety factor of less than 100 is justified. If the data on individuals showing a high level of conversion are used, a safety factor of 10 would be justified because intraindividual differences have already been taken into account.

Since uncertainties still exist with respect to the possible endogenous formation of *N*-nitroso compounds after nitrate exposure, the most appropriate approach at present is to derive an ADI based on the most sensitive toxicity criteria for nitrite in rats and the toxicokinetics of nitrate in humans, in addition to deriving an ADI directly from toxicity studies with nitrate.

On the basis of the NOEL of 370 mg of nitrate ion per kg of body weight per day in the long-term toxicity study in rats and a safety factor of 100, an ADI of 0–5 mg per kg of body weight, expressed as sodium nitrate, or 0–3.7 mg per kg of body weight, expressed as nitrate ion, could be allocated. On the basis of the NOEL of 160 mg per kg of body weight per day for normally responding individuals in the human population (5% (mol/mol) rate of conversion) and a safety factor of 50, an ADI of 0–3.2 mg per kg of body weight, expressed as nitrate ion, could be allocated. Both ways of deriving an ADI for nitrate thus give similar figures. The Committee therefore retained the previous ADI of 0–3.7 mg per kg of body weight, expressed as nitrate ion. This ADI is expressed to two significant figures because rounding up was not considered to be justified in view of the value of 3.2 mg per kg of body weight derived from data on the conversion of nitrate to nitrite. Because nitrate may be converted to nitrite in significant amounts and infants below the age of 3 months are more vulnerable to the toxicity of nitrite than adults, the ADI does not apply to such infants.

In deriving an ADI for nitrate the Committee took a cautious position. It was aware that vegetables are an important potential source of intake of nitrate. However, in view of the well-known benefits of vegetables and the lack of data on the possible effects of vegetable matrices on the bioavailability of nitrate, the Committee considered it inappropriate to compare exposure to nitrate from vegetables directly with the ADI and hence to derive limits for nitrate in vegetables directly from it.

Submission of the results of studies in humans exposed to nitrate from different sources (vegetables and drinking-water), including the toxicokinetics and relevant toxicodynamic parameters such as thyroid function and adrenal cortex function, is desirable. The results should be analysed by means of physiologically based pharmacokinetic (PBPK) models.

A toxicological monograph summarizing relevant information from the previous monograph and the information that has become available since the previous evaluation was prepared. The existing specifications for potassium nitrate and sodium nitrate were revised.

3.2.3 *Potassium bromate*

Potassium bromate was evaluated as a flour-treatment agent at the seventh, twenty-seventh, thirty-third and thirty-ninth meetings of the Committee (Annex 1, references *7, 62, 83* and *101*). At its thirty-third meeting (Annex 1, reference *83*), the Committee endorsed the recommendation made in previous reports that, as a general principle, bromate should not be present in food *as consumed.* This principle would also apply to other possible uses of potassium bromate in food processing, e.g. in treating barley in beer-making. At its thirty-ninth meeting (Annex 1, reference *101*), the Committee concluded that potassium bromate was a "genotoxic carcinogen" on the basis of the results of long-term toxicity/carcinogenicity studies and *in vivo* and *in vitro* mutagenicity studies. On the basis of these data and data on residual bromate in bread, the Committee also concluded that the use of potassium bromate as a flour-treatment agent was not appropriate. Consequently, the previous acceptable level of bromate for use in the treatment of flours for bread-making was withdrawn.

At its present meeting, the Committee was informed that new, more sensitive methods of analysis using gas chromatography/mass spectrometry (GC/MS) and inductively coupled plasma-mass spectrometry (ICP-MS) have been developed for the determination of bromate in bread, and that bromate residues have been detected in bread made from bromate-treated flour (*16*). No new toxicity data were available. Since potassium bromate is genotoxic and carcinogenic, and residues may be present in bread resulting from its use in flour, the Committee considered that the conclusions of the thirty-ninth meeting still apply.

A toxicological monograph was not prepared. The existing specifications for potassium bromate were revised.

3.3 Contaminants

3.3.1 *Ochratoxin A*

Ochratoxin A is a mycotoxin produced by a variety of species of the genera *Aspergillus* and *Penicillium*. It is found mainly in cereals and cereal products, some pulses, coffee, cocoa, figs, nuts and coconut products, but

can also occur in meat and dairy products derived from animals exposed to ochratoxin A-contaminated feedstuffs.

Ochratoxin A was first evaluated at the thirty-seventh meeting of the Committee (Annex 1, reference *94*), when a provisional tolerable weekly intake (PTWI) of 112 ng per kg of body weight was established. The assessment was based on the deterioration of renal function in pigs, for which the lowest-observed-effect level was 0.008 mg per kg of body weight per day (a no-effect level was not observed). A safety factor of 500 was used in deriving the tolerable intake of ochratoxin A. At that time, the Committee recommended that efforts should be made to highlight the need for ensuring proper storage conditions for grain and grain products. Furthermore, appropriate ochratoxin A residues should be monitored to obtain better estimates of dietary exposure and to identify populations at greater risk with a view to implementing preventive measures. The Committee also encouraged further studies aimed at elucidating 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.

In view of the increasing number of reports on the occurrence of ochratoxin A in food commodities in several countries, the Committee was asked to re-evaluate this substance.

Since the last review a number of toxicological studies have been conducted, including investigations on epidemiology, genotoxicity and nephrotoxicity. Although the results of these studies are important for understanding the biological effects of ochratoxin A, the Committee did not consider that they justified any change in the basis on which the previous assessment of the tolerable intake of ochratoxin A was made. In addition, the Committee confirmed that nephrotoxicity was the most sensitive effect of ochratoxin A and that the increased incidence of both benign and malignant tumours seen in the rat occurred at higher doses.

The Committee reconfirmed the PTWI established at the thirty-seventh meeting, rounded it off to 0.1 µg per kg of body weight, and reiterated its request for further studies on ochratoxin A.

The Committee noted that grain should be stored under suitable conditions to keep levels of ochratoxin A to a minimum.

An addendum to the toxicological monograph was prepared.

3.3.2 *Patulin*

Patulin is a mycotoxin produced by certain species of the genera *Aspergillus* and *Penicillium,* including *A. clavatus, P. expansum, P. patulum, P. aspergillus* and *P. byssochlamys. P. expansum* is a common spoilage microorganism in apples, and the major potential dietary sources of patulin are apples and apple juice made from affected fruit.

Patulin was previously evaluated by the Committee at its thirty-fifth meeting (Annex 1, reference *88*), when a PTWI of 7 µg per kg of body weight was established, based on a no-effect level of 0.1 mg per kg of body weight per day in a combined reproductive toxicity/long-term toxicity/carcinogenicity study in rats. Additional information has become available since the last evaluation.

Patulin was reviewed by the International Agency for Research on Cancer in 1976 and 1985 (*17, 18*). It was concluded at the second of these reviews that there was inadequate evidence for carcinogenicity of patulin in experimental animals. No evaluation could be made of carcinogenicity of patulin in humans.

In rats, most of the administered dose was eliminated within 48 hours in faeces and urine, less than 2% being expired as carbon dioxide. No other metabolites have been identified. About 2% of the administered dose was still present after 7 days, located mainly in erythrocytes.

Patulin has a strong affinity for sulfhydryl groups, which explains why it inhibits the activity of many enzymes. Patulin adducts formed with cysteine were less toxic than the unmodified compound in acute toxicity, teratogenicity, and mutagenicity studies.

In acute and short-term studies, patulin caused gastrointestinal hyperaemia, distension, haemorrhage and ulceration. Pigtail monkeys (*Macaca nemestrina*) tolerated patulin consumption of up to 0.5 mg per kg of body weight per day for 4 weeks without adverse effects.

The NOEL in a 13-week toxicity study performed in rats was 0.8 mg per kg of body weight per day, based on a slight impairment of kidney function and a villous hyperaemia in the duodenum in the mid- and high-dose groups.

Two reproductive toxicity studies in rats and teratogenicity studies in mice and rats were available. No reproductive or teratogenic effects were noted in mice or rats at dose levels of up to 1.5 mg per kg of body weight per day. However, maternal toxicity and an increase in the frequency of fetal resorptions were observed at higher levels, which indicated that patulin was embryotoxic.

Both *in vitro* and *in vivo* experiments indicated that patulin had immunosuppressive properties. However, the dose levels at which these effects occurred were higher than the NOEL in both the short-term toxicity study and a combined reproductive toxicity/long-term toxicity/carcinogenicity study.

Although the data on genotoxicity were variable, most assays carried out with mammalian cells were positive while assays with bacteria were mainly negative. In addition, some studies indicated that patulin impaired DNA synthesis. These genotoxic effects might be related to its ability to react with sulfhydryl groups and thereby inhibit enzymes involved in the replication of genetic material. Nevertheless, it was concluded from the available data that patulin is genotoxic.

The mortality seen in short-term toxicity, reproductive toxicity and long-term toxicity studies with conventional rats due to dilatation of the gut and/or pneumonia was most probably secondary to the fact that patulin acts like an antibiotic on Gram-positive bacteria, thereby giving a selective advantage to pathogenic Gram-negative bacteria. This conclusion was supported by the fact that, in 13-week studies at similar dose levels with specific pathogen-free (SPF) rats, no such mortality was seen.

In the combined reproductive toxicity/long-term toxicity/carcinogenicity study in rats, a dose level of 0.1 mg per kg of body weight per day of patulin produced no effect in terms of decreased weight gain in males. However, as patulin was administered only three times per week during 24 months, the NOEL derived from this study was 43 µg per kg of body weight per day.

An additional long-term carcinogenicity study in a rodent species other than the rat, which was recommended at the previous meeting for the further evaluation of the toxicity of patulin, was not available.

Since, in the most sensitive experiment, patulin was administered only three times per week, the existing PTWI was changed. As it does not accumulate in the body and in the light of the consumption pattern, the PTWI was changed to a provisional maximum tolerable daily intake (PMTDI). Based on a NOEL of 43 µg per kg of body weight per day and a safety factor of 100, a PMTDI of 0.4 µg per kg of body weight was established.

Submission of the results of a long-term toxicity/carcinogenicity study in a rodent species other than the rat is desirable.

Patulin levels in apple juice are generally below 50 µg per litre, and maximum intakes have been estimated to be 0.2 µg per kg of body weight per day for children and 0.1 µg per kg of body weight per day for adults, i.e. well below the tolerable intake established by the Committee. However, apple juice can occasionally be heavily contaminated, and continuing efforts are therefore needed to minimize exposure to this mycotoxin by avoiding the use of rotten or mouldy fruit.

A toxicological monograph, summarizing both the information given in the previous toxicological monograph and information received since the previous review, was prepared.

4. Revision of certain specifications

4.1 General

A total of 34 substances were examined for specifications only (see Annex 2). For 13 of these substances, no information was received to support the re-evaluation. The Committee was, however, able to revise the existing specifications for 31 substances, based on the substantial

efforts made by Committee members. In addition, the existing tentative specifications for propylene glycol alginate and sorbitan monolaurate were revised and the "tentative" qualification was deleted.

4.2 Gum arabic

The specifications for gum arabic were last revised at the thirty-fifth meeting of the Committee (Annex 1, reference *88*). At the present meeting, the Committee recognized that the previous revision led to an inconsistency in that the limits for optical rotation and nitrogen content would have the effect of excluding gums from certain types of *Acacia senegal*, and closely related species such as *Acacia seyal*. The tests for optical rotation and nitrogen content were therefore deleted. In revising the specifications, the Committee nevertheless fully took into account concerns expressed about the potential adulteration of commercial gum arabic with gums from non-*Acacia* species. It emphasized the importance of the Identification Test for Hydrolysis Products to ensure the absence of three sugars characteristic of common adulterants in gum arabic. The Committee also considered that gum arabic obtained from *A. senegal* or closely related species and meeting the revised specifications adequately reflects the materials that were toxicologically tested.

5. Future work

1. The Committee was aware that the Codex Committee on Food Additives and Contaminants, at its Twenty-seventh Session in March 1995, would consider appropriate recommendations on how to deal with 43 old identity and purity specifications which it had never reviewed. The Committee recognized the need for the revision of these specifications before they could be forwarded to the Codex Alimentarius Commission for adoption as Codex Advisory Specifications, and was prepared to examine them at future meetings as they are placed on the agenda.
2. The Committee noted that, since the publication of Environmental Health Criteria, No. 70 (Annex 1, reference *76*), there has been increased interest in neurotoxicity and immunotoxicity, areas which it has infrequently addressed. Greater attention should be paid to these areas in the future. In addition, there is a need for the Committee to provide further advice on the significance of findings in long-term toxicity studies and on the consequences of changing manufacturing processes.

6. Recommendations

1. In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, the important role that the recommendations of the Committee play in the development of

regulations in many countries, and the need for maintaining consistency and continuity within the Committee, 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. In view of the importance of identity and purity specifications in defining the products being considered in safety assessments, and their significance for the national food legislation of many FAO/WHO Member States, the Committee emphasized the need for the regular updating and compilation of the *Compendium of food additive specifications* published as FAO Food and Nutrition Paper, No. 52. The Committee recommended that FAO should consider publishing the second edition of the *Compendium* in CD-ROM and loose-leaf format to facilitate its use and timely updating.

3. The Committee was concerned that for many of the substances referred for re-examination of specifications there was little or no information provided in support of requests for changes and recommended that all interested parties should take steps to improve the situation.

4. The Committee recommended that, to enable it to plan its work more effectively, the Codex Committee on Food Additives and Contaminants should provide clear guidance on the priority to be given to substances to be re-evaluated, particularly bearing in mind the need for up-to-date evaluations of substances included in the General Standard for Food Additives.

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The Committee was saddened to hear of the death of Professor R. Truhaut, who was the person most responsible for the establishment of the Joint FAO/WHO Expert Committee on Food Additives. He was one of the most far-sighted toxicologists of the 1950s and was responsible for many of the operational procedures of the Committee. He regularly attended the Committee's meetings until recently, and was well known for his incisive comments and sense of humour. Although struggling against infirmities in his later years, he continued working effectively to the end.

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Annex 1

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Annex 2

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

Substance	Specifications[a]	Acceptable Daily Intake (ADI) in mg per kg of body weight and other toxicological recommendations
Antioxidants		
Butylated hydroxytoluene (BHT)	S	0–0.3
tert-Butylhydroquinone (TBHQ)	R	0–0.2 (temporary)[b]
Carrier solvent		
Diethylene glycol monoethyl ether	S	No ADI allocated[c]
Colour stabilizer		
4-Hexylresorcinol	N	Treatment of crustacea is not of toxicological concern[d]
Emulsifiers		
Dioctyl sodium sulfosuccinate	S	0–0.1
Glycerol ester of wood rosin	R	No ADI allocated[e]
Sucrose esters of fatty acids and sucroglycerides	R[f]	0–20 (temporary group ADI)[b, g]
Flavouring agent		
Ethyl vanillin	S	0–3
Food colours		
Canthaxanthin	R	0–0.03
Curcumin	S	0–1 (temporary)[b]
Glazing agents		
Microcrystalline wax[h]	R	0–20 (group ADI)[i]
Mineral oil (revised and divided into two groups):		
Mineral oil (high-viscosity)[j]	R	0–20
Mineral oil (medium- and low-viscosity)	R	
Class I[k]		0–1 (temporary)[b]
Class II[l] and Class III[m]		0–0.01 (temporary group ADI)[b]
Paraffin wax[n]	W	ADI withdrawn[o]
Sweetening agent		
Alitame	N	No ADI allocated[p]
Thickening agent		
Processed *Eucheuma* seaweed	R	0–20 (temporary)[b]
Miscellaneous substances		
β-Cyclodextrin	R	0–5
Nitrate	R[q]	0–3.7 (expressed as nitrate ion)[r]
Nitrite	R[s]	0–0.06 (expressed as nitrite ion)[t]
Potassium bromate	R	Use as a flour-treatment agent is not appropriate

Contaminant	Provisional Tolerable Intake in µg per kg of body weight
Ochratoxin A	Provisional Tolerable Weekly Intake (PTWI): 0.1
Patulin	Provisional Maximum Tolerable Daily Intake (PMTDI): 0.4

Substance (considered for specifications only)	Specifications[a]
Agar	R
Alginic acid	R
Ammonium alginate	R
Calcium alginate	R
Calcium propionate	R
Calcium stearoyl lactylate	R
Carmines	R
Carnauba wax	R
Carob bean gum	R
Carotenes (algae)	R
Carotenes (vegetable)	R
Citric acid	R
Cochineal extract	R
Diacetyltartaric and fatty acid esters of glycerol	R
Ethyl *p*-hydroxybenzoate	R
Guar gum	R
Gum arabic	R
Maltitol syrup	R
Methyl *p*-hydroxybenzoate	R
Microcrystalline cellulose	R
Nitrogen gas or liquid	R
Petroleum jelly	R,T[b]
Phosphoric acid	S
Polydextroses	R
Potassium alginate	R
Potassium propionate	R
Propionic acid	R
Propyl *p*-hydroxybenzoate	R
Propylene glycol alginate	R
Sodium alginate	R
Sodium propionate	R
Sodium stearoyl lactylate	R
Sodium thiocyanate	R
Sorbitan monolaurate	R

Notes to Annex 2

[a] 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.

[b] See Annex 3.

[c] In view of the apparent potential for significant exposure to this substance, and the absence of adequate long-term feeding studies, an ADI could not be allocated.

[d] Treatment of crustacea at concentrations of up to 50 mg/l, resulting in residue levels of approximately 1 mg/kg in the edible portion, is not of toxicological concern.

[e] An ADI could not be allocated because of the lack of adequate long-term toxicity/carcinogenicity and reproductive toxicity studies. The Committee considered that, as a minimum, studies demonstrating the metabolic stability and non-bioavailability of glycerol ester of wood rosin under conditions resembling those in the human gastrointestinal tract would be required to permit further evaluation of this material.

[f] Applies only to the specifications for sucrose esters of fatty acids. The specifications for the sucrose ester content of sucroglycerides were not reviewed.

[g] Temporary group ADI for the sucrose ester content of sucrose esters of fatty acids and sucroglycerides.

[h] Including HMPW (high-melting-point wax) and HSW (high-sulfur wax).

[i] Since both waxes fall within the same specifications, a group ADI was allocated to HMPW and HSW (see Annex 3).

[j] Including P100(H) oil.

[k] Including P70(H) oil.

[l] Including N70(H) and N70(A) oils.

[m] Including P15(H), N15(H) and N10(A) oils.

[n] Including LMPW (low-melting-point wax) and IMPW (intermediate-melting-point wax).

[o] The previous ADI "not specified" for paraffin wax was withdrawn, because toxicological effects were observed at all dose levels.

[p] An ADI could not be allocated because of deficiencies in the carcinogenicity studies in rats.

[q] The specifications were revised for both potassium nitrate and sodium nitrate.

[r] Because nitrate may be converted into nitrite in significant amounts and infants below the age of 3 months are more vulnerable to the toxicity of nitrite than adults, the ADI does not apply to such infants. The Committee was aware that vegetables are an important potential source of intake of nitrate. However, in the light of the well-known benefits of vegetables and the lack of data on the possible effects of vegetable matrices on the bioavailability of nitrate, the Committee considered it inappropriate to compare exposure to nitrate from vegetables directly with the ADI and hence to derive limits for nitrate in vegetables directly from it.

[s] The specifications were revised for both potassium nitrite and sodium nitrite.

[t] The ADI applies to all sources of intake. Nitrite should not be used as an additive in food for infants below the age of 3 months. The ADI does not apply to such infants.

Annex 3

Further toxicological studies and other information required or desired

Antioxidants

tert-Butylhydroquinone (TBHQ)

The final results of long-term toxicity studies in mice and rats that are known to have been completed are required for review in 1997.

Emulsifiers

Sucrose esters of fatty acids and sucroglycerides

The results of a well designed and conducted tolerance study in humans are required for review in 1997.

Food colours

Curcumin

The results of a reproductive toxicity study are required for review in 1998.

Glazing agents

Mineral oil (medium- and low-viscosity)

Information about the compositional factors in mineral oils that influence their absorption and toxicity is required for review in 1998. In addition, a study of at least 1 year's duration in F344 rats on one of these materials is required, which should include an assessment of immune function at appropriate time periods and an investigation of the kinetics of accumulation of the material, and particularly whether a plateau is reached. A reversal period of 1 year should also be included, in order to determine whether the granulomatous hepatic lesions observed in rats in the 90-day studies are fully reversible. The results of this study, together with all relevant background data, including the physical and chemical parameters of the materials tested, should be submitted for review in 1998.

Petroleum jelly

Information is required on methods of analysis and levels in commercial products, of viscosity at 100 °C, carbon number distribution at 5% distillation point, average relative molecular mass, and oil content.

Thickening agent

Processed Eucheuma seaweed

The results of a 90-day feeding study in rodents and an appropriate battery of genotoxicity studies on processed *Eucheuma* seaweed derived

from *E. cottonii*, all meeting present-day standards, are required for review in 1998.

If the specifications are to be expanded to include processed *Eucheuma* seaweed from *E. spinosum*, a 90-day feeding study in rodents and a separate battery of genotoxicity studies will be required on this material.

A complete review of all data on carrageenan should be undertaken in 1998, particular attention being paid to the identity of the source materials and to the specifications of the products that have been tested.

Miscellaneous substances

Nitrate

Submission of the results of studies in humans exposed to nitrate from different sources (vegetables and drinking-water), including the toxicokinetics and relevant toxicodynamic parameters such as thyroid function and adrenal cortex function, is desirable. The results should be analysed by means of physiologically based pharmacodynamic (PBPK) models.

Contaminants

Patulin

Submission of the results of a long-term toxicity/carcinogenicity study in a rodent species other than the rat is desirable.

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