EVALUATION OF CERTAIN FOOD ADDITIVES AND NATURALLY OCCURRING TOXICANTS

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

World Health Organization
Geneva 1992
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Joint FAO/WHO Expert Committee on Food Additives
Rome, 3–12 February 1992

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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 naturally occurring toxicants.* WHO Food Additives Series, No. 30, in press.

Specifications are issued separately by FAO under the title:


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**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 3 to 12 February 1992. The meeting was opened by Mr A.N. Cortas, Assistant to the Assistant Director-General, Economic and Social Policy Department, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr Cortas noted that the expert advice on and evaluations of food additives provided by the Committee formed a solid basis for the work of the Codex Alimentarius Commission and were referred to by governments, universities and industry around the world.

Mr Cortas drew attention to the gradual changes in the Committee’s functions and in the type of substances subjected to safety evaluation since the Committee’s inception in 1955. He noted that the Joint FAO/WHO Conference on Food Standards, Chemicals in Food and Food Trade, held in March 1991, had also made recommendations as to the future activities and objectives of the Committee (1). It was clear, therefore, that the role and importance of the Committee in food safety assessment could only increase and that the scope of its work might change again in the future.

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (2), there had been thirty-eight previous meetings of the Expert Committee (Annex 1). The present meeting was convened on the basis of the recommendation made at the thirty-seventh meeting (Annex 1, reference 94).

The tasks before the Committee were: (1) to further elaborate principles for evaluating the safety of food additives and naturally occurring toxicants (see section 2); (b) to undertake toxicological evaluations of certain food additives and naturally occurring toxicants (see section 3 and Annexes 2 and 3) and to review and prepare specifications for selected food additives; and (c) to discuss and advise on matters arising from the Twenty-third Session of the Codex Committee on Food Additives and Contaminants (see Annex 4).

2. **General considerations**

2.1 **Modification of the agenda**

Benzoin gum and polyglycerol esters of fatty acids were not considered because information was not available that would permit an evaluation of either substance.

Sucrose esters of fatty acids were added for evaluation together with sucroglycerides.

Chaconine was added for evaluation because it is closely related to solanine chemically and the two substances are normally both present in potatoes.
Hydrocarbon waxes were evaluated under the designation microcrystalline wax and paraffin wax, the substances for which specifications were established at the present meeting.

Cyanogenic glycosides and solanine, which had been placed on the agenda as contaminants, were considered by the Committee under the heading of naturally occurring toxicants.

2.2 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and naturally occurring toxicants, the Committee took into consideration the principles established and contained in WHO 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, and 94), including the present one. WHO 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 Use of safety factors

Safety factors have been used by the Committee since its inception to derive an Acceptable Daily Intake (ADI) for a given substance from a no-observed-effect level (NOEL) demonstrated in animal experiments. The most commonly used factor of 100 allows for differences between the test species and humans (10-fold) and differences between individuals (10-fold) (Annex 1, reference 76). However, safety factors have been used with considerable flexibility, ranging from 10 to several thousand, depending on the type and extent of the data as well as the nature of the toxicity.

The Committee was presented with a draft paper (3) in which it was proposed that each factor of 10 should be considered to comprise two aspects – pharmacokinetics and pharmacodynamics. Default values were proposed for each separate aspect, the product of which gave the original factor of 10. Where appropriate kinetic and dynamic data existed, these could be incorporated to replace the corresponding default values. The Committee considered that, because of a lack of appropriate data, there were a limited number of examples where the safety factor could be subdivided in this way. Further discussions would be necessary before more detailed consideration could be given to the matter by the Committee.
The Committee stressed its continuing interest in obtaining data related to the kinetic behaviour of food additives in humans and experimental animals and to mechanistic aspects, as well as data on interspecies and interindividual differences in these parameters. The Committee emphasized that it would continue to give such data – whenever available – appropriate consideration in the course of safety evaluations and that it encouraged future developments in this field (Annex 1, reference 94, section 2.2.1).

2.2.2 Flavouring substances

The Committee reconsidered the safety assessment of flavouring substances in the light of the special problems associated with this group of compounds, which arise from the large number concerned, their chemical diversity and, in many cases, the low levels of use. These special considerations dictate a degree of flexibility.

General principles for the safety assessment of food flavours are contained in Principles for the safety assessment of food additives and contaminants in food (Annex 1, reference 76) and were discussed at the thirty-fifth and thirty-seventh meetings of the Committee (Annex 1, references 88 and 94). At the thirty-fifth meeting, it was emphasized that a minimum amount of data was necessary to permit the development of a flexible procedure for evaluating these substances. At the thirty-seventh meeting, the Committee noted that the factors which should be considered in the safety evaluation of these substances included data from toxicological studies in animals and short-term tests for mutagenicity and clastogenicity, results of studies on metabolism and structure-activity relationships, the level of usage, the relative contribution of food additive use to total intake, the source of the flavouring agent, and data on the extent and frequency of human exposure (Annex 1, reference 94).

At its present meeting, the Committee noted that a flexible approach based on similar principles was being pursued by other organizations. It reiterated these principles and the conclusion that a minimum amount of data on the compound or a closely related material was necessary for safety evaluation. The precise amount of data required would depend on the nature of the chemical and information on exposure. The Committee also drew attention to the desirability of structurally related compounds being evaluated at the same time, since data derived from one member of a group of such compounds may assist in the evaluation of another (Annex 1, reference 94).

Data on structure-activity relationships and exposure, on which the system for allocating priorities for the evaluation of flavouring substances is based, are important in the safety evaluation of such compounds but are not by themselves sufficient for such an evaluation. Structure-activity relationships for safety evaluation purposes are relatively poorly developed but progress in this area may increase their value.
The Committee considered that the principles that had been developed provided an appropriate framework for a flexible approach to the safety evaluation of flavouring substances.

2.2.3 Evaluation of naturally occurring toxicants

The Committee was requested by the Codex Committee on Food Additives and Contaminants to review two naturally occurring toxicants, cyanogenic glycosides and solanine. Two other substances on the agenda, furfural and limonene, are also naturally present in many foods. It is likely that the Committee will be asked to review many more such substances because a high priority was given to the evaluation of naturally occurring toxicants at the Joint FAO/WHO Conference on Food Standards, Chemicals in Food and Food Trade (I, paragraphs 156 and 161 (iii)).

Cyanogenic glycosides are present in a number of foods, primarily some tuberous starchy crops such as cassava (manioc), nuts and fruit seeds such as almonds and stone-fruit (drupe) seeds, bamboo shoots, and certain species of beans. All are characterized by the release of hydrogen cyanide during preparation or digestion.

The response to cyanide depends both on the nutritional state of the consumer and on the frequency and magnitude of exposure. Populations that traditionally consume foods containing cyanogenic glycosides have learned by experience how to manage these potentially dangerous components of their diets. Likewise, commercial producers of foods such as tapioca, arrowroot flour, and marzipan are aware of the problem and generally ensure that the known dangers of poor food processing are controlled as far as possible so that potential exposure to cyanide is reduced to the lowest practical level.

Solanine or similar alkaloids are present in almost all solanaceous plants, the most important of which is the potato (Solanum tuberosum). There have been recorded instances where potatoes have produced intoxication in humans, but this has generally resulted from poor handling or storage practices. A notable exception was a new potato cultivar that was withdrawn from commerce because of the high levels of solanine in the tuber. As new cultivars are created, either through traditional plant-breeding practices or via bioengineering, those responsible should be aware of the possibility that such new solanaceous food crops may be toxic.

Traditionally, certain populations have learned to cope with a large number of toxicants occurring naturally in foods. As a generalization, it can be stated that there are many potentially unsafe foods but there are also safe ways of eating them. However, as new cultivars are developed in the search for commercial advantage, there may be concomitant increases in the content of naturally occurring toxicants to levels beyond those that can be dealt with by traditional practices. The Committee emphasized that food producers should be aware of this potential problem.
2.2.4 **Terminology**

Questions were raised at the Joint FAO/WHO Conference on Food Standards, Chemicals in Food and Food Trade (7) regarding the terminology used by the Committee. In particular, it was stated that the conditions for allocating an ADI “not specified” and the reasons for an ADI “not allocated” were often not clearly described in the annex to the report in which the results of the evaluations were summarized.

The Committee recognized that there were instances where it had not provided information on intake and/or use levels of food additives that had been allocated ADIs “not specified”. In some cases this was not necessary because foreseeable use was so self-limiting that it was extremely unlikely that intake would exceed the level of toxicological concern. Most enzyme preparations fall into this category. However, in other cases users had not had sufficient information to compare current uses of a food additive with those considered by the Committee, so that it had not been possible to determine whether intake exceeded the level considered at the time of the evaluation. The Committee therefore recommended that, when new uses that would significantly increase intake are envisaged, the food additive should be brought forward for re-evaluation. In the present report, uses have been listed when an ADI “not specified” has been allocated to a food additive and the use has not been considered to be self-limiting.

There are various reasons for not allocating an ADI, ranging from lack of information to data on adverse effects that call for advice that a food additive should not be used at all. In previous reports, the reasons for not allocating an ADI have been indicated in footnotes to the table in which the evaluation was summarized. At its present meeting, the Committee has used terminology that attempts to convey in a few words the reasons for not allocating ADIs. However, even with this terminology, the reader must refer to footnotes and to the report itself to gain a more complete understanding of the basis for the decision not to allocate an ADI.

Because of the complexities involved, it is difficult to describe the basis for the evaluations in a few words. The Committee therefore stressed the need for users to consult the sections of the report on specific substances rather than to rely solely on the summary in the annex. Officials responsible for establishing Codex standards and for regulating food additives and contaminants should also consult the toxicological monographs issued by WHO in the Food Additives Series and the specifications published by FAO in its Food and Nutrition Papers.

2.3 **Principles governing the establishment and revision of specifications**

2.3.1 **Preparation of specifications**

At the Third Joint FAO/WHO Conference on Food Additives and Contaminants, held in October 1973 (4), it was stated that the three main objectives of the specifications prepared by the Committee were:
1. To identify the substance that has been subjected to biological testing.
2. To ensure that the substance is of the quality required for safe use in foods.
3. To reflect and encourage good manufacturing practice.

The present Committee reaffirmed these principles.

2.3.2 Revision of the Guide to specifications

The Committee was informed that the revised Guide to specifications (Annex 1, reference 100) had been published. This new reference work was used by the Committee in preparing specifications at the present meeting.

3. Comments on specific food additives and naturally occurring toxicants

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

3.1 Specific food additives

3.1.1 Emulsifiers

Sucrose esters of fatty acids and sucroglycerides
Sucrose esters of fatty acids consist principally of mono-, di-, and small amounts of tri-esters of sucrose with fatty acids. They can be prepared from sucrose and the methyl and ethyl esters of fatty acids. Sucroglycerides consist essentially of mixtures of sucrose esters of fatty acids and mono- and diglycerides, and are produced by the reaction of edible fats or oils with sucrose.

These substances were previously evaluated by the Committee at its thirteenth, seventeenth, twentieth, twenty-fourth, and thirty-fifth meetings (Annex 1, references 39, 32, 41, 53, and 88). A group ADI of 0-10 mg per kg of body weight for sucrose esters of fatty acids and sucroglycerides was allocated at the twenty-fourth meeting; this was based on a no-effect level in rats of 1% in the diet, equivalent to 500 mg per kg of body weight per day, observed in a long-term study with sucrose monopalmitate, which had been reviewed at the thirteenth meeting of the Committee. A safety factor

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1 When the Committee's evaluations have included dietary studies, the levels of the substance administered to animals are often given in the form of percentages; these are calculated on a weight-for-weight basis, so that $0.1\% = 1 \text{ g/kg of feed}$, $1.0\% = 10 \text{ g/kg of feed}$, etc.
of 50 was applied because these substances were shown to be hydrolysed in the gut to normal food constituents prior to absorption.

At its thirty-fifth meeting, the Committee considered the use of various solvents in the manufacture of these substances, and also reviewed new studies on a palm-oil sucroglyceride, including a long-term carcinogenicity study in rats and short-term studies in rats and dogs. It was concluded that the previously established group ADI of 0–10 mg per kg of body weight for sucrose esters of fatty acids and sucroglycerides would apply both to sucrose esters of fatty acids manufactured by a process using various solvents and to the palm-oil sucroglyceride.

At its present meeting, the Committee was asked to re-evaluate sucroglycerides in order to clarify the relationship between them and sucrose esters of fatty acids. No new toxicological data were available.

The Committee noted that there were similarities in composition between the two materials in that both contain sucrose esters of fatty acids and mono- and diglycerides. The main difference is that sucroglycerides contain 40–60% mono- and diglycerides of fatty acids, while sucrose esters of fatty acids contain less than 20%. Mono- and diglycerides of fatty acids were evaluated by the Committee at its seventh and seventeenth meetings (Annex 1, references 7 and 32), when an ADI “not limited” (ADI “not specified” in current usage) was allocated because these compounds are normal constituents of the human diet and were therefore considered safe.

At its present meeting, the Committee concluded that studies performed on sucrose esters of fatty acids were valid for the evaluation of sucroglycerides, and vice versa.

The Committee noted that the in vivo and in vitro studies with sucrose mono-esters of fatty acids previously evaluated also included sucrose distearate and that this compound was hydrolysed in the gut (to sucrose and stearic acid) prior to absorption.

The Committee took the opportunity to re-evaluate the long-term study of sucrose monopalmitate in rats that formed the basis for the previously established no-effect level of 10 g/kg (1%) in the diet, equivalent to 500 mg per kg of body weight per day. In the earlier evaluation, records of lower body weight and lower food consumption were noted in the 3% test groups, the highest dose level employed. The Committee noted that only group means and the corresponding intragroup ranges were reported for body weight data and food consumption data; wide intragroup ranges were reported for body weights at the beginning of the study, making statistical evaluation of the results difficult because the possibility of obtaining statistically significant results by chance for specific time periods seemed to be high. The Committee concluded that food intakes and food utilization were lower in the dosed groups of both sexes, the high-dose groups being those most strongly affected, during the first one or two weeks of the study, and that this resulted in a tendency towards lower body weights throughout the study. This effect was most probably the result of
the decreased palatability of the test diets. As no other indications of toxicity were observed in the study at any dose level, the Committee was of the opinion that the 3% dose level, equivalent to 1.5 g per kg of body weight per day, represented the NOEL.

At its thirty-fifth meeting, the Committee reviewed new toxicological studies on a palm-oil sucroglyceride, including a long-term carcinogenicity study in rats and short-term studies in rats and dogs. From the long-term carcinogenicity study in rats, a NOEL for decreased body-weight gain of 5% in the diet, equal to 1.6 g per kg of body weight per day, was identified. At higher dose levels in both rats and dogs, weight gains were reduced and the serum concentrations of certain enzymes and of potassium ions rose, but fell again either during the study or on cessation of dosing. This NOEL was in line with the results of a number of previously evaluated short- and long-term toxicity studies performed in mice, rats, and dogs with a variety of sucrose esters of fatty acids and sucroglycerides, in some of which even higher NOELs had been obtained.

Recognizing the similarity between sucroglycerides and sucrose esters of fatty acids, the Committee concluded that these materials should be evaluated on the basis of their content of sucrose esters. On the assumption of a content of 50% of sucrose esters in the material used in the most recent long-term toxicity study in rats, the NOEL was 800 mg per kg of body weight per day.

As these compounds are hydrolysed in the gut to give normal dietary constituents prior to absorption, the Committee applied a safety factor of 50, to give a group ADI of 0-16 mg per kg of body weight for sucrose esters contained in sucrose esters of fatty acids and sucroglycerides. This ADI replaces the previous one.

The Committee stressed that this evaluation applied to sucroglycerides prepared from palm oil, lard, and tallow and to sucrose esters of fatty acids prepared from palmitic, stearic, and oleic acids, as well as palm oil, lard, and tallow. The Committee 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.

No toxicological monograph was prepared.

The existing specifications for sucroglycerides were maintained. The specifications for sucrose esters of fatty acids were not reviewed.

*Thermally oxidized soya bean oil (TOSO) and thermally oxidized soya bean oil interacted with mono- and diglycerides of fatty acids (TOSOM)*

Thermally oxidized soya bean oil (TOSO) is a complex mixture of substances formed by the thermal oxidation (190–250 °C) of refined soya bean oil. TOSOM is produced by reaction of TOSO with mono- and diglycerides of fatty acids (ratio 10:90) under vacuum at 130 °C.
TOSOM was considered at the fifteenth, seventeenth, twentieth, and twenty-fourth meetings of the Committee (Annex 1, references 26, 32, 41, and 53) under the name “esters of glycerol and thermally oxidized soya bean fatty acids”. A toxicological monograph was prepared at the seventeenth meeting, but an ADI was not established, pending the evaluation of short- and long-term studies on a material of well defined composition. TOSO was evaluated for the first time at the present meeting.

The Committee considered studies showing that, after oral administration of radiolabelled material to rats and mice, tissue levels of TOSOM were slightly greater than those of TOSO. The absorption of both thermally oxidized substances was lower than that of refined soya bean oil.

A 2.5-year study in rats used dietary levels of 3, 6, or 12% TOSOM (equal to 1.3, 2.7, and 5.4 g per kg of body weight per day in males; 1.8, 3.6, and 7.4 g per kg of body weight per day in females), and 0.3 or 1.2% TOSO (from two sources) (equal to 130 and 540 mg per kg of body weight per day in males; 180 and 740 mg per kg of body weight per day in females). The only effects noted were transient minor variations in total leukocyte counts and percentages of lymphocytes and monocytes, which were not considered to be of toxicological significance. For some sites, the incidence of tumours in treated animals was slightly higher ($P<0.05$) than in controls. However, the incidences were within the range of those of historical controls for Wistar rats, there was no dose-response relationship, and there was no consistency of effect for the three compounds tested. The Committee concluded that neither TOSO nor TOSOM is carcinogenic in rats.

Using a safety factor of 200, the Committee allocated an ADI of 0.3 mg per kg of body weight for TOSO and 0.3 mg per kg of body weight for TOSOM, based on the observation that the highest doses in the 2.5-year rat study (approximately 600 and 6000 mg per kg of body weight per day respectively) produced no adverse effects.

A toxicological monograph was prepared.

The existing tentative specifications for TOSOM were revised and retitled, and the Committee agreed to delete the “tentative” qualification. New specifications for TOSO were prepared.

3.1.2 Enzyme preparations

At its present meeting, the Committee evaluated three enzyme preparations. At its thirty-first meeting (Annex 1, reference 77), it had requested extensive toxicological data on cellulase from *Trichoderma longibrachiatum* (previously referred to as *T. reesei*) and β-glucanase from *Trichoderma harzianum* on the basis of the guidelines for enzyme preparations summarized in WHO Environmental Health Criteria, No. 70 (Annex 1, reference 76, Annex III).

At its present meeting, the Committee recognized that these guidelines
were oriented primarily towards enzyme sources and took insufficient account of intake levels. On the other hand, intake levels have been taken into consideration in evaluating certain other additives. The Committee concluded that there was good reason to give greater weight to intake in the evaluation of enzyme preparations, and did so in its reconsideration of these enzymes.

Cellulase derived from *Trichoderma longibrachiatum*

Cellulase derived from *Trichoderma longibrachiatum* (earlier referred to as *T. reesei*) was previously reviewed at the thirty-first meeting of the Committee (Annex 1, reference 77). The enzyme preparation is characterized by four primary enzyme activities: 1,4-β-D-glucan 4-glucanohydrolase (EC 3.2.1.4), 1,4-β-D-glucan glucohydrolase (EC 3.2.1.74), 1,4-β-D-glucan cellobiohydrolase (EC 3.2.1.91), and 1,3-β-D-glucan 3-glucanohydrolase (EC 3.2.1.6).

At the thirty-first meeting, the Committee reviewed 13-week studies in rats and dogs, reproduction and teratogenicity studies in rats, and studies on the mutagenicity of the enzyme preparation. A temporary ADI of 0-0.3 mg total organic solids (TOS) per kg of body weight was established by the application of a 2000-fold safety factor to the 2% NOEL (after conversion to TOS) in the 13-week study in rats. In accordance with Annex III of *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76), the Committee requested a long-term study in a rodent species. Inadvertently, a TOS of 31% instead of 87.8% was used when converting the 2% NOEL for the enzyme test preparation to TOS; thus the temporary ADI was determined to be 0-0.3 mg TOS per kg of body weight, whereas the true figure should have been 0-0.85 mg TOS per kg of body weight.

At its present meeting, the Committee reviewed two additional 13-week studies in rats in which no adverse effects were observed up to the highest dietary level (5%) tested. Since the potential intake was minimal and following a reconsideration of its guidelines for evaluating enzymes derived from microorganisms (see introduction to this section), the Committee concluded that it had been provided with sufficient information to establish an ADI “not specified” when the preparation is used in accordance with good manufacturing practice.

An addendum to the toxicological monograph was prepared. The existing tentative specifications were revised and the Committee agreed to delete the “tentative” qualification.

β-Glucanase from *Trichoderma harzianum*

β-Glucanase (EC 3.2.1.6) from *Trichoderma harzianum* was last evaluated at the thirty-first meeting of the Committee (Annex 1, reference 77), when a temporary ADI of 0-0.5 mg TOS per kg of body weight was established. At that time, the Committee considered data that showed no adverse effects in short-term studies in rats and dogs, no reproductive toxicity or
teratogenic effects in rats, no mutagenicity in bacteria or in the mouse lymphoma L5178Y cell assay, and no chromosomal damage in Chinese hamsters *in vivo*.

At the thirty-first meeting, the Committee considered that the source organism was neither a normal constituent of food nor a normal food contaminant. In accordance with its guidelines (Annex 1, reference 76, Annex III), the Committee therefore required the submission, by 1992, of a long-term study in a rodent species and additional information to show that the organism was not pathogenic to humans and did not produce antibiotics.

At its present meeting, the Committee noted that the *Trichoderma* were filamentous fungi that produced extracellular enzymes useful for food processing. It was evident that potential human intake would be minimal if the enzyme was used as specified in the production of wine.

On reconsidering its guidelines for evaluating enzymes derived from microorganisms (see introduction to this section), the Committee concluded that a long-term study would not now be required for this enzyme. The Committee also considered that the specifications were now adequate to demonstrate non-pathogenicity to humans and to exclude the production of antibiotics.

The Committee concluded that it had been provided with sufficient information to establish an ADI “not specified” for the enzyme when used in accordance with good manufacturing practice in wine-making.

No toxicological monograph was prepared. The existing tentative specifications were revised and the Committee agreed to delete the “tentative” qualification.

**Lysozyme**

Lysozyme has not previously been evaluated by the Committee. Egg-albumen lysozyme consists of 129 amino acids cross-linked by four disulfide bridges. Lysozymes have been found in animal tissues, organs, and serum as well as in tears, milk, and cervical mucus. Lysozyme hydrochloride is obtained commercially from edible hen egg-white by an ion-exchange process. The lysozyme concentration in egg albumen is about 5 g/kg.

Lysozyme (as the hydrochloride) is used in cheese production to prevent “late blowing”. This phenomenon is caused by the growth of *Clostridium tyrobutyricum*, which acts on the lactate resulting from the fermentation of lactose to produce carbon dioxide, hydrogen, butyric acid, and acetic acid. The use level is less than 40 mg of lysozyme per litre of cheese milk, resulting in a concentration of less than 400 mg of lysozyme per kg of cheese.

In studies related to allergic reactions, the reactions produced by egg-white lysozyme in animals and humans were less severe than those seen with
other proteins, e.g. ovalbumin and albumin, which have a long history of use as food components.

On the basis of the available data, the Committee concluded that the low additional intake of lysozyme via cheese was not a hazard to consumer health, provided that the enzyme complied with the specifications.

Lysozyme is obtained from edible animal tissue commonly used as food and can thus be designated as a class I enzyme and regarded as a food (Annex 1, reference 76; Annex III). It was therefore considered acceptable for use in food processing when used in accordance with good manufacturing practice.

A toxicological monograph was prepared. New specifications for lysozyme hydrochloride were prepared.

3.1.3 Flavouring substances

trans-Anethole

trans-Anethole was last reviewed by the Committee at its thirty-seventh meeting (Annex 1, reference 94), when it was noted that proliferative lesions occurred in the liver, with an increased incidence of hepatocellular adenomas and carcinomas in female rats at dietary levels of 1% (incorrectly stated as 10 mg/kg in the thirty-seventh report). At that time, the existing temporary ADI was reduced and extended to 1992, pending further metabolic and pharmacokinetic studies in mice, rats, and humans. In addition, it was stated that a long-term dietary study in mice might be needed. The Committee also concluded that chromosome aberration studies and in vitro tests for gene mutations in mammalian cells were desirable; the need for a reproduction/teratogenicity study would be considered by the Committee when further relevant data from the foregoing studies had been reviewed.

At its present meeting, the Committee was informed that studies designed to meet these requirements were under way, including comparative metabolic studies in mice and rats, studies on the effects of chronic dietary administration of trans-anethole on hepatic enzyme induction and cell proliferation in these species and on enzyme induction in humans, and in vitro cytotoxicity and genotoxicity studies. As these studies were incomplete, it was not possible for the Committee to evaluate them.

The Committee was of the opinion that a further chronic toxicity/carcinogenicity study in mice would be required, since the earlier study was inadequate and a no-effect level could not be established in the study in rats. Without an adequate chronic toxicity study, a comparison of the metabolism in mice and rats would have no toxicological meaning. In addition, the Committee considered that insufficient data were available to permit a final evaluation of the significance of the liver tumours observed in female rats with respect to the use of trans-anethole as a food additive. The remaining data available on trans-anethole were not deemed adequate for the allocation of a full ADI since the long-term study in mice was not of
a suitable standard, a NOEL could not be established in the long-term study in rats, and there was positive evidence of genotoxicity in in vitro bacterial mutation tests.

The existing temporary ADI of 0-0.6 mg per kg of body weight was established on the basis of the minimal-effect level of 125 mg per kg of body weight for non-neoplastic proliferative changes in the liver of rats, adjusted by using a safety factor of 200. The Committee extended the temporary ADI to 1997, pending the completion of the ongoing studies and the results of a long-term study in mice at appropriate levels to establish a no-effect level.

No toxicological monograph was prepared. The existing specifications were maintained.

**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. At its thirty-fifth meeting (Annex 1, reference 88), the Committee noted that none of the previously evaluated long-term studies met modern standards, 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 and metabolism studies in rats for evaluation in 1992.

At the present meeting, the Committee was informed that the studies requested had been initiated, and that preliminary results did not indicate any cause for concern. The main metabolite in rats seems to be ethyl vanilllic acid. This metabolite has also been identified in humans ingesting a vanilla-flavoured liquid supplementary diet.

The Committee extended the previously allocated temporary ADI of 0-5 mg per kg of body weight, pending submission of the final results of the ongoing short-term and metabolism studies in rats for evaluation by 1994.

No toxicological monograph was prepared. The existing specifications were revised.

**Limonene**

Limonene (±)-limonene or d-limonene) is a flavouring agent that has not been evaluated previously by the Committee.

(±)-Limonene has been shown to reduce body weights significantly in male and female mice and rats and in female rabbits. The NOEL for this effect was 150 mg of (±)-limonene per kg of body weight per day (administered by gavage) in a 2-year study in male rats.

Oral administration of (±)-limonene (400 mg per kg of body weight per day) to rats for 20 days slightly increased liver weight and phospholipid content, decreased liver and serum cholesterol levels, increased the concentration of cytochromes P-450 and b5, and increased the activities of
aminopyrine demethylase and aniline hydroxylase. Although liver lesions were not associated with the administration of (+)-limonene in a 2-year study in rats (doses up to 150 mg per kg of body weight per day for males; doses up to 600 mg per kg of body weight per day for females), a daily gavage dose of 500 mg of (+)-limonene per kg of body weight per day for 2 years was associated with an increased incidence of multinucleated liver hepatocytes and cytomegaly in male mice. The NOEL for these effects was 250 mg per kg of body weight per day, administered by gavage to male mice for 2 years.

Dermal application of (+)-limonene has been reported to cause irritation and immune-mediated skin reactions in a number of species, including humans. Oral administration of (+)-limonene to mice was reported to have immunological effects, including suppression of the in vitro mitogenic response by mouse T-cells and B-cells, and both suppression and stimulation of antigenic responses. However, the biological significance of the in vitro changes is not known.

Results of teratogenicity studies in mice suggest that maternal consumption of 2400 mg of (+)-limonene per kg of body weight per day, but not of 600 mg per kg of body weight per day; affected the development of fetuses (decreased body weight gain, increased incidences of lumbar rib and fused rib, and delayed ossification). However, (+)-limonene was reported to be non-teratogenic in rabbits at doses ranging from 250 to 1000 mg per kg of body weight per day (although the highest dose significantly reduced survival of the dams).

(+)-Limonene consistently produced negative results in genotoxicity studies, has been reported to inhibit the activity of other mutagens and carcinogens, and gave mixed responses in tumour-promotion studies.

Data clearly demonstrate that (+)-limonene exacerbates the spontaneously occurring nephropathy in mature male rats which, after prolonged exposure, is associated with an increase in the incidence of renal tumours that are not commonly observed in control male rats. However, (+)-limonene is not the only substance that has this effect: various hydrocarbon mixtures and several other chemicals have also been reported to exacerbate spontaneous nephropathy and cause renal tumours in male rats. Although, in studies designed to elucidate the mechanism of this carcinogenic process, chemicals other than (+)-limonene were generally used as test substances, such studies have shown that the following mechanism is likely to be applicable to (+)-limonene-induced nephropathy and renal tumours in male rats: (1) (+)-limonene and (+)-limonene-1,2-oxide (a metabolite of (+)-limonene) bind reversibly to $\alpha_2$-globulin in the kidneys of (+)-limonene-treated male rats; (2) this binding further slows down the normally slow processing of reabsorbed $\alpha_2$-globulin; and (3) as a result, $\alpha_2$-globulin accumulates in the phagolysosomes (hyaline droplets) of epithelial cells of the proximal convoluted tubules of the kidney, leading to necrosis, accumulation of cell debris, and regenerative hyperplasia. The postulated mechanism of action
for some non-genotoxic carcinogens suggests that regenerative hyperplasia may increase clonal expansion of spontaneously initiated cells and lead to the development of the renal tumours observed.

A NOEL for the (+)-limonene-associated increase in \( \alpha_2u \)-globulin levels in male rat kidney has not been identified, but the lowest-observed-effect level from a 21-day gavage study of (+)-limonene in male rats was 75 mg per kg of body weight per day. A sex-specific protein with molecular features similar to \( \alpha_2u \)-globulin has been identified in human urine, but the concentration is at least four orders of magnitude less than that of \( \alpha_2u \)-globulin in male rats.

The Committee concluded that the postulated mechanism for (+)-limonene-induced nephropathy and renal tumours in the male rat was probably not relevant to humans, and that toxic end-points associated with this effect were not appropriate bases for the derivation of an ADI for (+)-limonene.

Based on the significant decreases in body weight gain associated with administration of (+)-limonene to male and female mice and rats and female rabbits, an ADI of 0-1.5 mg per kg of body weight was established for this substance. The Committee considered the known natural occurrence and food additive uses of (+)-limonene, and concluded that only a small proportion of total intake is likely to be derived from direct additive use. The Committee therefore recommended that food additive intake be restricted to 75 \( \mu \)g per kg of body weight per day, which represents 5% of the maximum ADI for (+)-limonene.

A toxicological monograph and new specifications were prepared.

**Quinine**

Quinine was previously evaluated at the thirty-fifth meeting of the Committee (Annex 1, reference 88), when a temporary ADI was allocated. The effect of concern in normal humans was an acute, transient nystagmus. At its present meeting, the Committee reviewed further human data from a new study designed to determine the no-effect-level for acute effects. It also considered the results of a consumption study, conducted in France, Spain and the United Kingdom, on quinine from beverages.

The Committee concluded that the data demonstrated a clear NOEL with respect to the ocular effects of 80 mg of anhydrous quinine hydrochloride per day, equivalent to 72 mg of quinine free base. No treatment-related effects on audition or clinical biochemical abnormalities were observed at doses up to 160 mg of anhydrous quinine hydrochloride per day.

The previously established temporary ADI was withdrawn and, in the light of data on levels of use in beverages, the results of the consumption study and the additional data on humans, the Committee concluded that current use levels in soft drinks of up to 75 mg/l (as quinine base) were not of toxicological concern. However, the Committee noted that a small group
of consumers shows an idiosyncratic hyper-reactivity to quinine, and recommended that the consumer should be informed by appropriate means of the presence of quinine in foods and beverages in which it is used. The contribution of other uses of quinine in food and alcoholic beverages to daily intakes was considered to be negligible.

A monograph addendum was prepared. The existing specifications for quinine hydrochloride were revised. The existing specifications for quinine sulfate were not reviewed.

3.1.4 Solvents

1,2-Dichloroethane

1,2-Dichloroethane was evaluated at the fourteenth and twenty-third meetings of the Committee (Annex 1, references 22 and 50). At its thirty-fifth meeting (Annex 1, reference 89), in its deliberations on specifications for spice oleoresins, the Committee expressed the opinion that the use of 1,2-dichloroethane as an extraction solvent should be discouraged because of toxicological concerns, and recommended an overall review of chlorinated hydrocarbon solvents used in food processing.

At the present meeting, the Committee reviewed toxicological studies on 1,2-dichloroethane that had become available since its twenty-third meeting.

1,2-Dichloroethane is readily absorbed from the gastrointestinal tract after oral ingestion and via the lungs after inhalation. Following gastrointestinal absorption, radiolabelled 1,2-dichloroethane shows a preference for liver and adipose tissue but is readily metabolized and excreted as non-volatile metabolites in the urine and as volatile metabolites via exhalation. In studies in rats, 70-85% of an oral dose appeared in the urine as metabolites within 48 hours, 10-20% appeared in the exhaled air, partly as carbon dioxide, and small amounts were eliminated via the faeces or remained in the carcass at 48 hours irreversibly bound to macromolecules, mainly proteins. At high dose levels, the metabolism of 1,2-dichloroethane may become saturated. 1,2-Dichloroethane is more easily absorbed from the gastrointestinal tract when administered in aqueous rather than in oil solution.

The compound is able to cross the placental barrier of pregnant rats. However, no reproductive or teratogenic effects have been observed in inhalation studies in rats and rabbits.

Metabolism of 1,2-dichloroethane may occur via two pathways: one dependent on microsomal cytochrome P-450-mediated oxidation and the other on glutathione conjugation mediated by cytosolic glutathione S-transferases. The metabolism of 1,2-dichloroethane in vitro by microsomal mixed-function oxidases leads to the formation of 2-chloroacetalddehyde and 2-chloroethanol. 2-Chloroacetalddehyde may react with cellular macromolecules or undergo further metabolism to
2-chloroacetic acid, which is excreted in the urine either unchanged or as thioethers after conjugation with glutathione. Although the microsomal mixed-function oxidase pathway in vitro produces intermediates that bind to macromolecules, this pathway does not appear to be the most important in producing metabolites that are mutagenic in Salmonella typhimurium. In contrast, conjugation of 1,2-dichloroethane with glutathione is thought to lead to the formation of the mutagenic 2-chloroethyl glutathione and of ethylene. Of these, the former binds irreversibly to protein, DNA, and RNA and forms glutathione conjugates, which are excreted in the urine as thioethers. When the microsomal metabolism of 1,2-dichloroethane is inhibited, the glutathione-dependent metabolism increases, resulting in increased toxicity and carcinogenicity.

1,2-Dichloroethane is more toxic when given by a bolus gavage to rats than when given at corresponding doses in the drinking-water. In short-term studies in rats, the main target tissues were the liver, kidney, central nervous system, and forestomach. The first three of these are also the sites affected in humans accidentally exposed to high concentrations of the compound. No effects were observed when 1,2-dichloroethane was given orally to rats at 10 mg per kg of body weight 5 times per week for 90 days.

1,2-Dichloroethane has been shown to be carcinogenic in long-term studies in mice and rats following administration by gavage in corn oil at doses of 50-300 mg per kg body weight 5 days per week. In female mice, the compound induced mammary and uterine adenocarcinomas and possibly squamous-cell carcinomas of the forestomach, while hepatocellular carcinomas were induced in male mice. Lung adenomas and malignant histiocytic lymphomas where induced in mice of both sexes. When tested by inhalation, 1,2-dichloroethane was not carcinogenic in mice.

In the rat, 1,2-dichloroethane given by gavage in corn oil at time-weighted average doses of 47 and 95 mg per kg of body weight per day caused an increase in the total number of tumours in females only at the higher dose. In addition, an increased number of mammary-gland adenocarcinomas and fibroadenomas was seen in the females and of squamous-cell carcinomas of the forestomach in the males at the higher dose. An increase in the incidence of haemangiosarcomas seen in animals of either sex at both dose levels was statistically significant only for males. When tested in an inhalation experiment, 1,2-dichloroethane exposures of 5-150 mg/litre of air for 78 weeks did not significantly increase the tumour incidence in rats.

1,2-Dichloroethane was weakly mutagenic in Salmonella typhimurium TA1535 and TA100. Mutagenicity was enhanced by the addition of glutathione, and seemed to depend on cytosolic glutathione S-transferases. Mutagenic effects also occur in fungi, Drosophila spp., and mammalian cells in vitro. Neither micronuclei nor dominant lethal mutations were induced by 1,2-dichloroethane in mice, but a weak mutagenic effect was
reported in the mouse spot test. DNA damage, measured as unscheduled DNA synthesis in mammalian cells in vitro and as alkaline DNA unwinding in an in vivo/in vitro system, has been reported.

The Committee concluded that this compound has shown genotoxicity in both in vitro and in vivo test systems and that it is carcinogenic in mice and rats when administered by the oral route. No ADI was therefore allocated. The Committee expressed the opinion that 1,2-dichloroethane should not be used in food.

An addendum to the toxicological monograph was prepared but no specifications were prepared.

Dichloromethane

Dichloromethane was evaluated by the Committee at its fourteenth, twenty-third, and twenty-seventh meetings (Annex 1, references 22, 50, and 62).

At its twenty-seventh meeting, the Committee recommended that the use of dichloromethane as an extraction solvent should be limited in order to ensure that levels in foods are as low as practicable, since the available lifetime studies in rats and mice, because of a number of shortcomings, were felt to be inadequate for the possible carcinogenicity of this compound to be fully evaluated.

At its thirty-fifth meeting (Annex 1, reference 88), the Committee in its deliberations on specifications for spice oleoresins expressed the opinion that the use of dichloromethane as an extraction solvent should be discouraged because of toxicological concerns, and recommended an overall review of chlorinated hydrocarbon solvents used in food processing.

At the present meeting, the Committee reviewed additional toxicological data that had become available since the twenty-seventh meeting.

Dichloromethane is readily absorbed from the gastrointestinal tract and distributed to the blood, liver, lung, kidneys, body fat, and nervous tissues of animals and humans. The absorption proceeds at a faster rate when the compound is ingested in water solution than in oil solution. The compound is readily cleared from the organism, mainly by exhalation of the parent compound and the metabolites carbon dioxide and carbon monoxide. The formation of these metabolites is dose-dependent; at higher doses (exceeding 100 mg per kg of body weight), a proportionally higher level is expired as the parent compound. Systemic accumulation of dichloromethane does not occur.

Dichloromethane is able to cross the placental barrier in pregnant rats, but no reproductive effects have been observed even at high doses.

Dichloromethane is metabolized to carbon monoxide and carbon dioxide by two pathways, one dependent on oxidation by mixed-function oxidases and the other on glutathione S-transferases. The mixed-function oxidase
pathway seems to be the preferred metabolic route at low concentrations of dichloromethane, while at higher concentrations this pathway becomes saturated, making a larger percentage of dichloromethane available for metabolism by the glutathione-dependent pathway.

The metabolic production of carbon monoxide from dichloromethane leads to the formation of carboxyhaemoglobin, the cause of the hypoxic state commonly seen in accidental poisoning by dichloromethane.

Single and repeated doses of dichloromethane have produced elevations in serum enzyme levels indicative of kidney and, in particular, liver toxicity. No adverse effects were seen in rats in a 3-month study when dichloromethane was given orally at approximately 230\text{mg} per kg of body weight per day. Slight hepatocellular changes were noted in rats at oral doses administered via the drinking-water of 420-607\text{mg} per kg of body weight per day for 90 days.

High doses of dichloromethane are neurotoxic, the neurotoxicity depending on both a direct, non-specific narcotic action on the central nervous system, and an equally non-specific carbon monoxide-induced hypoxic effect. A variety of behavioural effects, such as increased motor activity and decreased learning ability, have been observed in experimental animals after inhalation exposure to high concentrations of dichloromethane.

Dichloromethane is weakly mutagenic in *Salmonella typhimurium* tester strain TA100, while mainly negative results have been produced in other tester strains. Weak clastogenic effects have been recorded in mammalian cell-culture systems *in vitro*, while tests for point mutations and DNA interactions have mainly failed to show any effects, in agreement with DNA-binding studies in rodents, in which dichloromethane-DNA adducts have not been detected after *in vivo* treatment with dichloromethane. When *in vivo* systems have been used, e.g. tests for unscheduled DNA synthesis in liver, sister chromatid exchange, and chromosomal aberrations in bone marrow of mice, dichloromethane has not produced effects when given orally, but has done so in a number of studies after inhalation of high doses.

In a long-term study in mice in which dichloromethane was administered in the drinking-water, there was a slight dose-related increase in fatty infiltrations in the liver of male and female mice given the highest dose of 250\text{mg} per kg of body weight per day. This effect was not reported in mice receiving a dose of 185\text{mg} per kg of body weight per day. A slight, but statistically significant, dose-related increase in combined hepatocellular carcinomas and adenomas in male mice was found to be within the incidence range of historical controls. In another long-term study in mice, in which dichloromethane was administered by gavage in olive oil at daily doses up to 500\text{mg} per kg of body weight, no liver carcinogenicity was observed. However, the treatment produced excess mortality, and the exposure had to be terminated after 64 weeks. When the mortality was
taken into account, there was a slight but significant increase in the incidence of pulmonary tumours in the males given the highest dose. In a study of similar design in rats in which dichloromethane was administered by gavage in olive oil at daily doses up to 500 mg per kg of body weight, the exposure also had to be terminated after 64 weeks because of excess mortality. In this study, no statistically significant increases in tumour incidences were seen.

When high doses of dichloromethane have been administered by inhalation to mice over the entire lifetime, increased incidences of lung tumours (alveolar/bronchiolar adenomas and carcinomas) and liver tumours (hepatocellular adenomas and carcinomas) have been reported, and in three different lifetime studies where dichloromethane was administered to rats by inhalation exposure at high concentrations (500 mg/litre or higher), an increased incidence of benign mammary-gland tumours (adenomas, fibromas, fibroadenomas) was seen in females. In one of the studies, adenocarcinomas were also found, together with a positive trend in the incidence of benign tumours in the mammary-gland area in males.

New long-term studies in mice and rats on the carcinogenicity of dichloromethane administered by the oral route were either negative or inconclusive because of premature deaths. As regards the administration of dichloromethane by the inhalation route, the available studies in mice and rats point to a carcinogenic effect on the liver and lung of mice and the mammary gland of rats receiving high doses.

A physiologically based pharmacokinetic model that provides quantitative data on the rates of metabolism and levels of dichloromethane in various organs was applied to the dose levels used in the above-mentioned long-term studies in mice and rats. It was calculated that the concentrations of glutathione-dependent metabolites in the liver and lungs of the mice that received the material in the drinking-water study were several orders of magnitude lower than those in the mice receiving dichloromethane in the inhalation study. In addition, the model predicted that considerably lower concentrations of these metabolites would be present in the liver and lungs of the rats exposed to dichloromethane in the inhalation studies than in the mice similarly exposed. This may provide an explanation for the differences in the results obtained in carcinogenicity studies in which different routes of administration are used.

Epidemiological studies have not shown any carcinogenic effect of dichloromethane after occupational exposure. However, the Committee noted that the power to detect excess risk in these studies was limited.

On the basis of the available data, the Committee concluded that the use of dichloromethane as an extraction solvent in food processing should be limited to use for spice oleoresins and the decaffeination of tea and coffee, and for food additives in which previous specifications drawn up by the Committee included residues of dichloromethane. The Committee was
made aware that stabilizers may be used in dichloromethane, and was of 
the opinion that only those that are toxicologically acceptable, and 
therefore not expected to lead to toxicologically significant residues, 
should be used in food-grade dichloromethane.

An addendum to the toxicological monograph was prepared. Existing 
specifications were revised but maintained as “tentative”.

*Diethylene glycol monoethyl ether*

This substance was previously reviewed at the twentieth meeting of the 
Committee (Annex 1, reference 47) but no ADI was allocated because of 
inadequacies in the toxicological data. In particular, the lack of metabolic 
and reproduction/teratogenicity studies was noted, and the carcinogenic-
ity studies did not meet current standards. At its present meeting, the 
Committee reviewed new data on metabolism and excretion in humans, 
together with studies on reproduction and teratogenicity in mice and 
rats, and a number of genotoxicity studies that were generally negative. 
It was also aware of a number of other studies on pharmacological 
activity and irritancy.

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, but no data on potential daily 
intakes were available. In these circumstances, the principles previously 
developed for materials occurring in foods in small amounts (Annex 1, 
reference 76) were not applicable. Although the metabolism, reproduction 
and teratogenicity, and genotoxicity studies met some of the requirements 
of the Committee, information on chronic toxicity/carcinogenicity was still 
inadequate. In view of the apparent potential for relatively high exposure to 
this substance, the Committee was unable to allocate an ADI because of 
the lack of such information.

In order to re-evaluate diethylene glycol monoethyl ether, the Committee 
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 an adequate carcinogenicity/chronic study in rats and 
mice.

The Committee considered that, in the light of the data reviewed at the 
present meeting, the 6-month study in pigs requested at its twentieth 
meeting would not be needed for a re-evaluation.

A toxicological monograph was prepared. The existing tentative 
specifications were revised and the Committee agreed to delete the 
“tentative” qualification.
3.1.5 Thickening agents

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

These substances were evaluated at the seventh and seventeenth meetings of the Committee (Annex 1, references 7 and 32). At the seventeenth meeting, an ADI of 0-50 mg per kg of body weight was established. Since these evaluations, additional data have become available and were reviewed by the Committee at its present meeting.

Alginate solutions of different viscosities are used as texture modifiers in a wide variety of food and industrial applications.

Three limited long-term dietary studies, one in mice and two in rats, provided no indication of a carcinogenic effect of alginites. Neither an in vitro nor an in vivo genotoxicity study showed any genotoxic activity. No effects on the reproduction of rats were observed, but the experimental design of the study was limited. A long-term study in mice using only a single dose level of 25% sodium alginate in the diet showed a clear effect (soft stool, distended caecum, decreased growth and deposition of calcium in the pelvis of the kidney).

In a 90-day study in rats, 15% sodium alginate in the diet resulted in an enlarged, distended, heavy caecum, a papillomatous appearance in the urinary bladder and calcium deposits in the renal pelvis and/or renal papilla. A slight decrease in growth was seen in only one short-term experiment with rats at the 10% level, but the effects were not specific and were also seen with other poorly absorbed compounds. Recent studies have shown slight interference with the absorption of a number of minerals.

The Committee recalled the similar effects of poorly absorbed compounds (modified celluloses, polyalcohols, gums, modified starches) reviewed in section 2.2.3 of the report of the thirty-fifth meeting (Annex 1, reference 58); for such compounds an ADI “not specified” had usually been allocated. The Committee therefore allocated a group ADI “not specified” to alginic acid and its ammonium, calcium, potassium, and sodium salts, but pointed out (as it had for certain other compounds) that laxative effects might occur at a high level of intake.

Propylene glycol alginate was evaluated at the Committee’s seventeenth meeting (Annex 1, reference 32), when an ADI of 0-25 mg per kg of body weight was allocated. It was not re-evaluated at the present meeting.

A toxicological monograph was prepared.

Each of the existing specifications for alginic acid and its ammonium, calcium, potassium, and sodium salts was revised. The Committee also took the opportunity to revise the existing specifications for propylene glycol alginate and designated them as “tentative”.

Processed Eucheuma seaweed

At its thirtieth meeting (Annex 1, reference 73), the Committee was asked
to evaluate semirefined carrageenan, which it named “processed *Eucheuma* seaweed” because this name described the true nature of the product. While recognizing that simple processed seaweeds were used as foods in certain parts of the world, the Committee was unable to evaluate processed *Eucheuma* seaweed and assign an ADI to it, as it did not comply with the specifications for carrageenan, for which toxicological data were available and to which an ADI “not specified” was allocated at the twenty-eighth meeting (Annex 1, reference 66). No toxicological data on processed *Eucheuma* seaweed were available to the Committee at the thirtieth meeting.

Since the previous review, no new toxicological studies have been reported. The Committee was only able to consider processing and compositional data provided since the previous review. These indicated that the processed product should not be regarded as a food.

The composition of processed *Eucheuma* seaweed also differs significantly from that of carrageenan in terms of the content of acid-insoluble material composed of cellulose, protein and lipids derived from the cell wall. The precise nature and toxicological significance of these compositional differences are unclear, so that the data used in the evaluation of carrageenan could not be used by the Committee as the sole basis for the evaluation.

In the absence of relevant toxicological data, processed *Eucheuma* seaweed could not be evaluated for use in foods. The Committee was aware that a 90-day study in rats was about to be undertaken, which might assist in a future evaluation of this material.

Tentative specifications had been drawn up at the thirtieth meeting of the Committee (Annex 1, reference 73) to assist in the identification of appropriate materials to be used in further toxicological studies. These specifications do not adequately describe the current product and no longer serve their original purpose. The Committee therefore decided to revise them, but to maintain the “tentative” qualification, on the understanding that new specifications would be prepared later if deemed necessary in the light of new toxicological information. In revising the specifications, the Committee considered the substance name. Since the revised specifications serve the same purpose as the original specifications, namely to provide a description of the substance to be used in toxicological studies, the name “processed *Eucheuma* seaweed” was retained.

If new specifications are prepared to describe the substance tested toxicologically, the Committee will again consider the substance name. The manufacturer currently calls this product “alternatively refined carrageenan”. The substance name must meet the criteria set out in the Committee’s thirty-third report (Annex 1, reference 83), section 2.3.4 “Guidelines for designating titles for specifications monographs”.

No toxicological monograph was prepared.
3.1.6 Waxes

**Beeswax**

Beeswax has not been previously evaluated by the Committee. The only data available to the Committee indicated that the LD$_{50}$ (median lethal dose) in the rat was greater than 5 g per kg of body weight per day and that beeswax was not mutagenic when tested in *in vitro* microbial assays.

The Committee concluded that beeswax could be regarded as a food constituent and that, although an evaluation in the traditional manner could not be carried out, the long history of use of natural yellow beeswax without apparent adverse effects provided a degree of assurance that its present functional uses (release and glazing agent in bakery products, glazing agent on fresh and frozen fruit, glazing agent on candy, carrier for flavours, and component of chewing-gum bases) did not raise any toxicological concerns.

The processing necessary to obtain bleached white beeswax did not appear to alter this conclusion, as the specifications limit the levels of peroxides present.

The Committee noted that beeswax might have allergenic potential and that the consumer should be made aware of its presence in foods.

The Committee also noted that attention should be paid to the possibility that toxic substances present in honey in some parts of the world might also occur in beeswax.

A toxicological monograph and new specifications were prepared.

**Candelilla wax**

Candelilla wax has not been previously evaluated by the Committee, which reviewed a number of older studies in mice, rats, and dogs. A 6-month study in dogs and a 2-year study in rats, with the highest dietary levels equivalent to 600 and 750 mg per kg of body weight per day, respectively, showed no compound-related toxicity. These studies were considered to be of fundamental importance in the safety assessment. In addition, microbial tests for mutagenicity were negative.

The Committee felt that the deficiencies noted in the individual studies, particularly in the light of current criteria, were counterbalanced to some extent by the consistent absence of adverse effects, and concluded that the present functional uses (glazing agent, component of chewing-gum base, surface-finishing agent, and carrier for flavour) did not raise any toxicological concerns.

A toxicological monograph and new specifications were prepared.

**Carnauba wax**

Carnauba wax has not been evaluated previously by the Committee.

Short-term feeding studies of carnauba wax at 10% in the diet of rats
showed no significant compound-related toxic effects. In a 28-week study with beagle dogs in which carnauba wax was fed at levels of 0.1, 0.3, and 1% in the diet, no compound-related toxic effects were identified.

No adverse effects were observed in fetuses in a teratogenicity study in rats following dietary exposure to 0.1, 0.3, and 1% carnauba wax during gestation.

A reproduction study combined with a 13-week oral toxicity study with F₁ animals was conducted in Wistar rats. During both phases of the study, animals (parents and offspring) were fed diets containing 0.1, 0.3, or 1% carnauba wax. No adverse effects associated with the consumption of carnauba wax at levels up to 1% of the diet (equal to 700 mg per kg of body weight per day for female rats) were observed in this experiment.

Although the results of mutagenicity studies were largely negative, scattered positive responses with *S. typhimurium* strains TA1537 and TA1538 were observed in the presence of metabolic activation.

The Committee allocated an ADI of 0-7 mg per kg of body weight for carnauba wax.

A toxicological monograph and new specifications were prepared.

*Microcrystalline wax and paraffin wax*

Food-grade petroleum-derived hydrocarbon waxes were first evaluated at the thirtieth meeting of the Committee (Annex 1, reference 73). At that time, the Committee was informed that toxicity data were available on certain hydrocarbon waxes, including the results of long-term feeding studies in rats, but noted that these studies were carried out with hydrocarbon waxes that had been in commercial use in the 1960s. Because the Committee was not informed whether the hydrocarbon waxes tested in these long-term studies were equivalent to those currently produced by both traditional and newer processes, no ADI was established.

At the thirty-third meeting (Annex 1, reference 83), the Committee reiterated its need to be informed whether the chemical composition of paraffin wax in current use met specifications for this substance. It decided that, for newer formulations of paraffin wax, new specifications were required, and that adequate long-term, mutagenicity, and reproduction/teratogenicity studies should be completed (Annex 1, reference 83).

At the present meeting, the Committee concluded that waxes tested in previous studies contained a broader spectrum of waxes than those in use today; two specifications for food-grade petroleum-derived hydrocarbon waxes were prepared (paraffin wax and microcrystalline wax; see final paragraph of this section). Because these specifications limit the number of waxes that can be used for food applications as compared with those tested in previous studies, the Committee concluded that previous long-term toxicity studies were suitable for evaluating the safety of hydrocarbon
waxes in current use. Additional long-term and mutagenicity studies on paraffin wax and microcrystalline wax were therefore not required.

The results of extraction and migration tests performed on waxes or wax-bearing products indicated that hydrocarbon waxes consumed in the diet are not absorbed or metabolized in significant amounts.

Five groups of 50 6-8-week-old male and female Sprague-Dawley rats were fed diets containing 10% ground wax (petrolatum; 3 samples) for 2 years. In addition, 157 female and 140 male rats served as untreated controls. Waxes were chosen such that the range of contents of polycyclic aromatic hydrocarbons represented that of waxes in commercial use (0-0.64 mg/kg). The rats were observed and weighed every other week, and all gross lesions were recorded. Rats were observed until spontaneous death or were killed in extremis; necropsies were performed on all animals, and all abnormal tissues were subjected to histological examination. Survival rates and average weights of experimental groups did not differ significantly from those of control animals and the incidence of tumours observed in the experimental animals was consistently similar to that of the same tumours in controls. No other wax-associated toxic effects were identified histologically. In particular, the effects seen in recent 90-day studies on mineral oils – deposition in the reticuloendothelial system and granulomas in the livers of rats – were not observed in the 2-year study of waxes in rats.

A series of 180-day feeding studies in rats were performed over a period of approximately 15 years (beginning in 1955) on chewing-gum bases containing hydrocarbon wax in proportions varying from 2% to 57% of the gum base. Calculated feeding levels for the waxes varied from 0.16% to 4.75% of the diet. Reported results included information on body weight, food consumption, urine composition, and gross pathological and histopathological changes. No compound-related effects were observed either in the studies taken as a whole, or in those in which hydrocarbon wax accounted for a high percentage of the chewing-gum base.

Five petrolatum waxes were negative for local and systemic carcinogenicity or toxicity in skin-painting studies in mice and rabbits. However, wax disc implants, but not ground wax implants, were associated with the development of fibrosarcomas at the implantation site in rats.

Because long-term toxicity studies indicated that petroleum-derived paraffin and microcrystalline waxes are non-toxic and non-carcinogenic, the Committee established a group ADI “not specified” 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.

A toxicological monograph was prepared.
The Committee reviewed the existing tentative specifications for paraffin wax in the light of currently available information. It was recognized that the name “paraffin wax” could be misleading as a name for the entire group of petroleum-derived waxes. These, besides paraffin wax, also include “intermediate wax” and “microcrystalline wax”. It was also noted that there were distinct differences in both chemical and physical properties between microcrystalline wax and the other petroleum-derived waxes. The Committee therefore decided to prepare two separate sets of specifications for paraffin wax (including intermediate wax) and microcrystalline wax.

Shellac
Shellac has not been previously evaluated by the Committee. It has a long history of use in food coatings and there are no reports attributing adverse effects to such uses.

As evaluated by microbial assays, shellac is not mutagenic. In a 90-day study, female rats fed diets containing 2% shellac showed caecal enlargement and swelling in the proximal region of the colon. There were no histopathological changes associated with the consumption of shellac, although the results were confounded by the failure of both experimental and control animals to grow during the latter part of the study.

A recent 90-day study (including an in utero exposure phase) produced no evidence of treatment-related toxic or pathological effects in F0 or F1 rats fed diets containing up to 1% shellac (equal to 660 mg per kg of body weight per day for female rats).

The Committee concluded that the present functional uses of shellac (as a coating, glazing, and surface-finishing agent applied externally to food) were of no toxicological concern.

A toxicological monograph and new tentative specifications were prepared.

3.1.7 Miscellaneous substances

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, 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 evaluation of new data (Annex 1, references 47, 53, 59, and 73).

At the thirtieth meeting, the Committee concluded that turmeric was often
regarded as a food rather than a food additive, and it was therefore not appropriate to allocate an ADI to this substance.

At the thirty-fifth meeting, the Committee reviewed curcumin and turmeric oleoresin separately and the temporary ADI for curcumin was extended until 1992, pending the results of long-term carcinogenicity studies initially requested at the twenty-sixth meeting. At that time, the Committee was informed that carcinogenicity studies, including fertility-assessment phases, were under way in rodents given turmeric oleoresin containing a high concentration of curcuminoids. The results of these long-term studies have not been submitted.

Long-term studies on curcumin were originally requested because data reviewed at the twenty-second and twenty-sixth meetings indicated that extracts of turmeric caused chromosomal aberrations in in vitro assays in mammalian and plant cells and inhibited fertility in rats. At its present meeting, the Committee was informed that the results of the carcinogenicity studies of turmeric oleoresin would be available later in 1992, but that these studies would not provide information about the reproductive toxicity/teratogenicity of curcumin. The amount of curcumin in the test substances was not known to the Committee.

The temporary ADI for curcumin of 0–0.1 mg per kg of body weight was extended. The results of the carcinogenicity studies in mice and rats given turmeric oleoresin (which are known to have been completed) and the results of a reproduction/teratogenicity study with curcumin are required for evaluation by 1995.

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

Furfural
Furfural has not been previously evaluated by the Committee.

Furfural occurs naturally and is formed during the processing and domestic preparation of a broad range of foods. It is also carried over into food from its use as an extraction solvent or as a component of flavour mixtures. Food additive or processing use provides an intake estimated to be no more than 0.5–1% of the intake from other food sources.

The Committee considered data on the absorption, metabolism, acute and chronic toxicity, genotoxicity, and carcinogenicity of furfural.

Furfural is absorbed by all routes of exposure. It is metabolized by oxidation to furoic acid and by subsequent conjugation with glycine. The half-life in humans is 2.0–2.5 hours.

The liver is the primary target for furfural toxicity in rats and mice. In short-term studies, it produced liver enlargement at doses $\geq 90$ mg per kg of body weight per day in rats and at 75–300 mg per kg of body weight per day in mice. At higher doses it produced centrilobular necrosis and cholangiofibrosis.
In a 2-year gavage study, using dose levels of 0, 50, 100, or 175 mg per kg of body weight per day in B6C3F1 mice and 0, 30, or 60 mg per kg of body weight per day in F344/N rats, furfural induced a statistically significant increase in the incidence of hepatocellular adenomas and carcinomas in male mice (34-64% compared with 32% in controls), hepatocellular adenomas in female mice (6-16% compared with 2% in controls), and bile-duct dysplasias (4%) plus cholangiocarcinomas (4%) in male rats at the highest dose level (compared with zero incidence in controls and a historical control incidence of 3 in 2145 (0.14%) for cholangiocarcinomas). While furfural was generally negative in bacterial mutagenicity tests, it was positive in a range of other tests for genotoxicity. The pattern of oncogene expression in liver tumours in furfural-treated mice differed from that in the spontaneous liver tumours of the controls.

While taking into consideration the relatively high concentrations of furfural in some foods as normally prepared and consumed, the Committee considered that it could not allocate an ADI to furfural because of the evidence of genotoxicity and carcinogenicity. The Committee considered that its direct addition as a flavour was not appropriate, and that its use as a solvent should be restricted to situations when alternatives were not available, e.g. for the purification of food oil by extraction of unsaturated components. Carry-over into food should be reduced to the lowest extent technologically feasible.

A toxicological monograph was prepared.

The existing specifications for this substance were revised, and designated as “tentative” pending information on the use of the substance as an extraction solvent.

**Potassium bromate**

Potassium bromate is used in treating barley in beer-making in addition to its use in the treatment of flour for bread-making.

Potassium bromate was evaluated as a flour-treatment agent at the seventh, twenty-seventh, and thirty-third meetings of the Committee (Annex I, references 7, 62, and 83), when the general principle was reiterated that bromate should not be present in foods as consumed, and that the use of potassium bromate could only be approved in such circumstances. At the thirty-third meeting, the Committee reduced the acceptable level for flour-treatment in bread-making to 60 mg/kg, taking account of the fact that: (a) bromide resulting from flour-treatment with potassium bromate at levels of ≤ 60 mg/kg did not present a toxicological hazard; and (b) residue data indicated that no detectable levels of bromate remained in bread baked from flour treated with bromate at levels up to 62.5 mg/kg. Because of the lack of residue data, no acceptable treatment levels could be established for food other than flour intended for baking.

Since then, new toxicological data have become available. Recent oral long-term toxicity/carcinogenicity studies of potassium bromate revealed
renal-cell tumours, peritoneal mesotheliomas, and thyroid follicular-cell tumours in rats and a slightly increased incidence of renal-cell tumours in hamsters. In view of these findings and the results obtained in in vivo as well as in vitro mutagenicity studies, it was concluded that potassium bromate is a genotoxic carcinogen. Experiments using new sensitive methods have also demonstrated that, when it is used for flour-treatment at what were regarded as acceptable levels, bromate is nevertheless present in bread.

On the basis of the new safety data and the new data on residual bromate in bread, the Committee concluded that the use of potassium bromate as a flour-treatment agent was not appropriate. The previous acceptable level of treatment of flours for bread-making was therefore withdrawn. The Committee was aware that alternatives were available. It was unable to address the use of potassium bromate in beer-making owing to the lack of data on its levels in beer.

An addendum to the toxicological monograph was prepared. The existing specifications were revised to delete the functional use of potassium bromate as a flour-treatment agent and were made “tentative”. Information on other uses was requested.

3.2 Naturally occurring toxicants

3.2.1 Cyanogenic glycosides

The cyanogenic glycosides, which are found in at least 2000 plant species, have not been evaluated previously by the Committee. There are approximately 25 known cyanogenic glycosides, some of which occur in edible parts of plants used for human and animal consumption, such as amygdalin in almonds, dhurrin in sorghum, linamarin and lotaustralin in cassava and lima beans, prunasin in stone-fruit, and taxiphyllin in bamboo shoots. While the cyanogenic glycosides differ considerably in bioavailability, those absorbed intact from the gut are not biotransformed to hydrogen cyanide by mammalian enzymes.

The potential toxicity of a cyanogenic plant depends primarily on its capacity to produce a concentration of hydrogen cyanide toxic to animals and humans. The release of hydrogen cyanide can occur either following maceration of the plant material – this activates the intracellular β-glucosidase which in turn hydrolyses glycoside – or by hydrolysis of glycoside by the β-glucosidase produced by the microflora of the gut. The level of β-glucosidase activity in the gut depends on the pH and the bacterial composition. The cyanogenic glycoside content of a foodstuff, when known, is usually expressed in terms of the amount of cyanide released by acid hydrolysis; exact figures for the concentration of the glycosides themselves are very rarely given.

Hydrogen cyanide absorbed from the gut can be detoxified by metabolic conversion to thiocyanate; this depends on the presence of nutritional factors, such as sulfur-containing amino acids and vitamin B₁₂. Acute
toxicity results when the rate of absorption of hydrogen cyanide is such that the metabolic detoxification capacity of the body is exceeded. The potential problems arising from chronic exposure have not been fully resolved.

Available reports of toxicological studies lack information on the level of intake of cyanogenic glycosides or on the amount of hydrogen cyanide potentially released. No long-term toxicity or carcinogenicity studies were available to the Committee. However, in vitro and in vivo genotoxicity studies were negative. Teratogenic and adverse reproductive effects attributable to linamarin (cassava) and hydrogen cyanide were seen only at doses that also caused maternal toxicity.

The toxic effects of cyanide on the thyroid (via its metabolite thiocyanate) depend on the iodine status of the test animals, as indicated in the twenty-fifth report of the Committee (Annex 1, reference 56).

On the basis of epidemiological observations, associations have been made between chronic exposure to cyanogenic glycosides and diseases such as spastic paraparesis, tropical ataxic neuropathy, and goitre. However, these observations were confounded by nutritional deficiencies, and causal relationships have not been definitely established.

Traditional users of foods containing cyanogenic glycosides usually have a basic understanding of the treatment required to render them safe for consumption. However, some products are sold commercially and are consumed by people who may not be familiar with such procedures. The Committee therefore recommended that guidelines be developed to provide reliable and sensitive methods for the analysis of these foodstuffs for hydrogen cyanide releasable from cyanogenic glycosides, in order to ensure that amounts in foods as consumed do not present a hazard.

Because of a lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glycosides could not be estimated. However, the Committee concluded that a level of up to 10 mg/kg hydrogen cyanide, as specified in the Codex Standard for Edible Cassava Flour (5), is not associated with acute toxicity.

A toxicological monograph was prepared.

3.2.2 Solanine and chaconine

A number of glycoalkaloids occur in plants of the Solanaceae family such as potatoes and tomatoes. The Committee was asked to evaluate solanine, one of the principal glycoalkaloids in potatoes.

Potatoes normally provide the main source of α-solanine, and since this occurs in potatoes together with a closely related glycoalkaloid, α-chaconine, the Committee decided to consider both substances together. α-Solanine and α-chaconine have the same basic alkaloid structure and differ only in the structure of the carbohydrate side-chain (Fig.1).
The content of $\alpha$-solanine and $\alpha$-chaconine in potatoes is normally about 20–100 mg/kg, although sunlight, mechanical damage, blight, sprouting, processing, and storage can all result in increases in the glycoalkaloid level and, in extreme cases, can cause the tuber to have a bitter taste. The skin and eye regions of the tuber normally contain the highest concentrations of glycoalkaloids, which are not destroyed by cooking.

Numerous studies performed on a variety of experimental animal species to elucidate the toxicological properties of glycoalkaloids, including teratogenicity, were evaluated.

Cranial abnormalities have been observed in some teratogenicity studies with laboratory animals, particularly with the hamster at levels of 165–200 mg of glycoalkaloids per kg of body weight per day. However, the suggested association of the consumption of blighted potatoes during pregnancy with increased incidences of spina bifida and anencephaly has not been substantiated.

In a limited study in humans, the daily consumption of potato tubers
containing approximately 24 mg of glycoalkaloids per 100 g did not result in any signs of acute toxicity. However, human poisonings have been associated with the consumption of poor-quality potato tubers with elevated levels of glycoalkaloids. The signs of low-grade glycoalkaloid poisoning are acute gastrointestinal upset with diarrhoea, vomiting, and severe abdominal pain. In more severe cases, neurological symptoms, including drowsiness, apathy, confusion, weakness, and vision disturbances followed by unconsciousness, have also been reported.

The Committee considered that, despite the long history of human consumption of plants containing glycoalkaloids, the available epidemiological and experimental data from human and laboratory animal studies did not permit the determination of a safe level of intake. The Committee recognized that the development of empirical data to support such a level would require considerable effort. Nevertheless, it felt that the large body of experience with the consumption of potatoes, frequently on a daily basis, indicated that normal glycoalkaloid levels (20–100 mg/kg) found in tubers that have been properly grown and handled were not of concern. To support the continued safe use of potato tubers, those developing new cultivars, and others growing, harvesting, storing, processing, and consuming potatoes, should be aware of the possibility of inadvertently increasing the content of glycoalkaloids to potentially toxic levels.

4. **Revision of certain specifications**

4.1 **General**

A total of 22 substances were examined for specifications only (see Annex 2), and the specifications for 16 were revised. The existing tentative specifications for the remaining six substances (anoxomer, carbon dioxide, ethyl hydroxyethyl cellulose, lactitol, mixed carotenoids, and sulfur dioxide) were maintained because insufficient information was received to revise them and remove the “tentative” qualification.

For four substances (aluminium powder, carthamus red, sorbitan monolaurate, and talc), some of the information requested by the Committee (Annex 1, references 68, 71, and 75) was not received. The Committee therefore revised the existing tentative specifications for all four, but maintained the “tentative” qualifications.

The Committee considered eight substances with existing tentative specifications (calcium stearoyl lactylate, carotenoids (algae), carotenes (vegetable), dammar gum, sodium stearoyl lactylate, sorbitan tristearate, tannic acid, and titanium dioxide) and concluded that sufficient information was now available to revise these specifications and to delete the “tentative” qualifications.
4.2 Addendum to the general specifications for enzymes used in food processing

At the thirty-seventh meeting of the Committee (Annex 1, reference 94), a draft addendum to the general specifications for enzymes used in food processing was prepared. This addendum addressed the new concerns relating to enzyme preparations from genetically modified organisms, which were being evaluated at that time. At the present meeting, such substances were not considered, but comments on the addendum provided by the Association of Microbial Food Enzyme Producers were reviewed. No modifications to the addendum were made, pending receipt of further comments from other interested parties. The Committee encouraged all who had an interest in enzymes produced by genetically modified organisms to provide written comments and suggestions. The addendum would be published in the Compendium of food additive specifications (Annex 1, reference 96), which would contain the combined specifications from the first to the thirty-seventh meetings of the Joint FAO/WHO Expert Committee on Food Additives (1956-1990).

5. Future work

1. The Committee reviewed five sorbitan esters (the mono-esters of palmitic, stearic, oleic, and lauric acids and the tri-ester of stearic acid) at its twenty-sixth meeting (Annex 1, reference 59). Since then, some of the specifications have been revised, but now that newer assay methods are available the Committee considered that all five specifications should be redrafted on a common basis at a future meeting and that, where necessary, further information should be sought to enable this to be done.

2. During its evaluation of specifications, the Committee noted that the heavy metals limit test currently used for most specifications might not be an adequate general screening procedure for heavy metals, as this test was insensitive to several metals of concern (e.g. cadmium and mercury). It therefore recommended that this method be reviewed at a future meeting and possible alternative, more sensitive and/or specific methods be considered.

3. During its evaluation of the specification for dammar gum, the Committee noted that the original specifications differed markedly from new data provided by manufacturers. This suggested that older specifications of other compounds should also be reviewed to ensure that they reflected current practices in the manufacturing and food-processing industries.
6. **Recommendations**

1. In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, 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. The Committee recommended that guidelines be developed to provide reliable and sensitive methods of analysing foodstuffs for hydrogen cyanide releasable from cyanogenic glycosides, in order to ensure that amounts in foods as consumed do not present a hazard.

3. The Committee recommended that, when plant cultivars that have the potential to produce cyanogenic glycosides and glycoalkaloids are developed, attempts be made to limit the content of these toxicants as much as possible. This requirement may need to be balanced against the possible protection afforded to the plant by these substances.

4. The Committee took note of the extensive pharmacokinetic and mechanistic studies undertaken with dichloromethane which, although they did not completely solve the problem of the chemical identity of the carcinogenic metabolites, did point to the potential utility of data of this type in safety evaluations. The Committee recommended that such studies continue to be undertaken, and encouraged efforts to employ physiologically based pharmacokinetic modelling to assist in the toxicity assessment of compounds under review.

5. Recognizing the problems associated with obtaining toxicological monographs and addenda that have been published over a number of years, some of which are out of print, the Committee recommended that WHO should consider consolidating and reissuing the monographs and/or making them available in electronic format.

6. The Committee noted with approval and appreciation the production of a catalogue, entitled “World Health Organization reports on food additives, food contaminants, and veterinary drug residues in food”, which listed all the reports, toxicological monographs, and specifications resulting from meetings of the Joint FAO/WHO Expert Committee on Food Additives and issued by WHO. It recommended that every effort should be made to keep the listing up to date. One possibility might be for all future reports of the Committee to contain a supplement sheet that could be incorporated into the catalogue. If this was done, an update of the complete catalogue would be required at no more than 10-year intervals.

7. When a substance is scheduled for evaluation by the Committee, a data sheet is prepared containing information such as data on the manufacturing process, intake levels, and fate in foods. This valuable information is used during the evaluation and portions may be
incorporated in the meeting report or other documents published later. The data sheets themselves, however, are not published. The Committee recommended that these data sheets be retained so that the information could be made available and updated in conjunction with future evaluations of the same substance, due attention being paid to the need to protect trade secrets.

Acknowledgements

The Expert Committee wishes to thank: Dr R. Cabral, Scientist, International Agency for Research on Cancer, Lyon, France; Dr K. Tanaka, Office of Environmental Chemicals Safety, Environmental Health Bureau, Ministry of Health and Welfare, Tokyo, Japan; and Professor M.M. Younes, WHO European Centre for Environment and Health, Bilthoven, Netherlands, for their valuable contributions to the meeting.

References


3. Renwick AG. Data derived safety factors and the regulation of food additives and environmental chemicals. Food additives and contaminants, in press.


Annex 1

**Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives**


4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Expert Committee). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives, vol. II. Food colours*. Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).


63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No.18, 1983.

64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No.28, 1983.


### Annex 2

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

<table>
<thead>
<tr>
<th>Substance</th>
<th>Specifications</th>
<th>Acceptable Daily Intake (ADI) in mg per kg of body weight and other toxicological recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emulsifiers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose esters of fatty acids and sucroglycerides</td>
<td>S</td>
<td>0–16 (group ADI)²</td>
</tr>
<tr>
<td>Thermally oxidized soya bean oil</td>
<td>N</td>
<td>0–3</td>
</tr>
<tr>
<td>Thermally oxidized soya bean oil interacted with mono- and diglycerides of fatty acids</td>
<td>R</td>
<td>0–30</td>
</tr>
<tr>
<td><strong>Enzyme preparations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulase from <em>Trichoderma longibrachiatum</em></td>
<td>R</td>
<td>Not specified³</td>
</tr>
<tr>
<td>β-Glucanase from <em>Trichoderma harzianum</em></td>
<td>R</td>
<td>Not specified³</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>N</td>
<td>Acceptable for use in food processing⁴</td>
</tr>
<tr>
<td><strong>Flavouring substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>trans</em>-Anethole</td>
<td>S</td>
<td>0–0.6 (temporary)⁵</td>
</tr>
<tr>
<td>Etyyl vanillin</td>
<td>R</td>
<td>0–5 (temporary)⁵</td>
</tr>
<tr>
<td>Limonene</td>
<td>N</td>
<td>0–1.5⁶</td>
</tr>
<tr>
<td>Quinine hydrochloride</td>
<td>R</td>
<td>Current use levels up to 75 mg/l (as quinine base) in soft drinks not of toxicological concern</td>
</tr>
<tr>
<td>Quinine sulfate</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td><strong>Solvents</strong></td>
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<td></td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>None</td>
<td>ADI not allocated because of evidence of genotoxicity and carcinogenicity</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>R, T⁵</td>
<td>Should be limited to current uses⁷</td>
</tr>
<tr>
<td>Diethylene glycol monoethyl ether</td>
<td>R</td>
<td>ADI not allocated because of inadequate data</td>
</tr>
<tr>
<td><strong>Thickening agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alginic acid and its ammonium, calcium, potassium, and sodium salts</td>
<td>R</td>
<td>Not specified (group ADI)³</td>
</tr>
<tr>
<td>Processed <em>Eucheuma</em> seaweed</td>
<td>R, T⁵</td>
<td>ADI not allocated because of inadequate data</td>
</tr>
<tr>
<td>Substance</td>
<td>Specifications</td>
<td>Acceptable Daily Intake (ADI) in mg per kg of body weight and other toxicological recommendations</td>
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<tr>
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<td>-----------------------------------------------------------------------------------------------</td>
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<tr>
<td><strong>Waxes</strong></td>
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<td></td>
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<tr>
<td>Beeswax</td>
<td>N</td>
<td>Present uses not of toxicological concern[8]</td>
</tr>
<tr>
<td>Candelilla wax</td>
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<td>Present uses not of toxicological concern[9]</td>
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<tr>
<td>Carnauba wax</td>
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<tr>
<td>Microcrystalline wax</td>
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<td>Not specified (group ADI)[3, 10]</td>
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<tr>
<td>Paraffin wax</td>
<td>R</td>
<td>Not specified (group ADI)[3, 10]</td>
</tr>
<tr>
<td>Shellac</td>
<td>N, T^5</td>
<td>Present uses not of toxicological concern[11]</td>
</tr>
<tr>
<td><strong>Miscellaneous substances</strong></td>
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<td></td>
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<tr>
<td>Curcumin</td>
<td>R</td>
<td>0–0.1 (temporary)[9]</td>
</tr>
<tr>
<td>Furfural</td>
<td>R, T^6</td>
<td>ADI not allocated because of evidence of genotoxicity and carcinogenicity[12]</td>
</tr>
<tr>
<td>Potassium bromate</td>
<td>R, T^5</td>
<td>Not appropriate for use as a flour-treatment agent</td>
</tr>
<tr>
<td><strong>Naturally occurring toxicants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solarine and chaconine</td>
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<td>Safe level of intake could not be estimated because of a lack of quantitative data</td>
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<td>Normal levels in potatoes (20–100 mg per kg of potatoes) not of toxicological concern</td>
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<table>
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<tr>
<th>Substance (considered for specifications only)</th>
<th>Specifications</th>
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<tr>
<td>Aluminium powder</td>
<td>R, T^5</td>
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<tr>
<td>Anoxomer</td>
<td>S, T^9</td>
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<tr>
<td>Calcium stearoyl lactylate</td>
<td>R</td>
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<tr>
<td>Carbon dioxide</td>
<td>S, T^5</td>
</tr>
<tr>
<td>Carob bean gum</td>
<td>R</td>
</tr>
<tr>
<td>Carotenes (algae)</td>
<td>R</td>
</tr>
<tr>
<td>Carotenes (vegetable)</td>
<td>R</td>
</tr>
<tr>
<td>Carthamus red</td>
<td>R, T^5</td>
</tr>
<tr>
<td>Dammar gum</td>
<td>R</td>
</tr>
<tr>
<td>Ethyl hydroxyethyl cellulose</td>
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<tr>
<td>Isomalt</td>
<td>R</td>
</tr>
<tr>
<td>Lactitol</td>
<td>S, T^5</td>
</tr>
<tr>
<td>Mixed carboxymethyl cellulose</td>
<td>S, T^5</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>R</td>
</tr>
<tr>
<td>Pectins</td>
<td>R</td>
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<tr>
<td>Substance (considered for specifications only)</td>
<td>Specifications¹</td>
</tr>
<tr>
<td>---------------------------------------------</td>
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</tr>
<tr>
<td>Sodium stearoyl lactylate</td>
<td>R</td>
</tr>
<tr>
<td>Sorbitan monolaurate</td>
<td>R, T³</td>
</tr>
<tr>
<td>Sorbitan tristearate</td>
<td>R</td>
</tr>
<tr>
<td>Sulfur dioxide</td>
<td>S, T³</td>
</tr>
<tr>
<td>Talc</td>
<td>R, T³</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>R</td>
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<tr>
<td>Titanium dioxide</td>
<td>R</td>
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</table>

**Notes to Annex 2**

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

2. Based on sucrose esters contained in sucrose esters of fatty acids and sucroglycerides.

3. ADI "not specified" means that, on the basis of the available data (chemical, biochemical, toxicological, and other), the total daily intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary.

4. This enzyme is derived from edible animal tissue commonly used as food; it may therefore be regarded as food.

5. See Annex 3.

6. Applies to total intake of limonene. Food additive intake should not exceed 75 μg per kg of body weight per day, which represents 5% of the maximum ADI.

7. Should be limited to use as an extraction solvent for spice oleoresins and the decaffeination of coffee and tea, and for food additives in which previous specifications drawn up by the Committee included residues of dichloromethane.

8. Beeswax is used as a release and glazing agent in bakery products, a glazing agent on fresh and frozen fruit, a glazing agent on candy, a carrier for flavours, and a component of chewing-gum bases.

9. Candelilla wax is used as a glazing agent, a component of chewing-gum base, a surface-finishing agent, and a carrier for flavouring substances.

10. Group ADI for microcrystalline and paraffin waxes for uses listed in the specifications, (chewing-gum base, protective coating, defoaming agent, and surface-finishing agent).

11. Shellac is used as a coating, glazing, and surface-finishing agent externally applied to food.

12. The direct addition of furfural to food as a flavouring substance is not appropriate and its use as a solvent should be restricted to situations in which alternatives are not available, e.g. for the purification of food oil by extraction of unsaturated components.
Annex 3

**Further toxicological studies and other information required**

**Flavouring agents**

*trans-Anethole*

The results of ongoing metabolic and pharmacokinetic studies in mice, rats, and humans and the results of a long-term toxicity study in mice are required for evaluation by 1997.

*Ethyl vanillin*

The results of ongoing short-term toxicity and metabolic studies in rats are required for evaluation by 1994.

**Solvents**

*Dichloromethane*

Information is required on the nature, level(s), and method(s) of analysis of stabilizers in food-grade dichloromethane. More information is also required on the assay for dichloromethane, including the analytical method used.

**Thickening agents**

*Processed Eucheuma seaweed*

Information is required on actual levels of arsenic, heavy metals, and lead occurring as contaminants in the commercial product and on the effect of cellulose removal in a viscosity test.

**Waxes**

*Shellac*

Information is required on the acid value, conditions for loss during the drying test, melting range, specific gravity (relative density), iodine value, and saponification value.

**Miscellaneous substances**

*Curcumin*

The results of carcinogenicity studies on mice and rats given turmeric oleoresin (which are known to have been completed) and the results of a reproduction/teratogenicity study with curcumin are required for evaluation by 1995.

*Furfural*

Information is required on specifications for the use of furfural as an extraction solvent.
Potassium bromate
Information is required on uses in food other than as a flour-treatment agent, the levels of such use, and the resultant residues in food.

Specifications only

Aluminium powder
Information is required on methods of production.

Anoxomer
Additional information is required on molecular weight distribution and confirmation is required that the analytical methods referred to actually determine the phenols in the bound state within the polymer.

Carbon dioxide
Information is required on the adequacy of the test method for oil content.

Carthamus red
Information is required on the absorptivity of carthamus red as used in the assay calculation.

Ethyl hydroxyethyl cellulose
Information is required on methods of analysis and on the limits (where applicable) for ethylene oxides, 1,4-dioxane, and ethylene chlorohydrin (2-chloroethanol).

Lactitol
Information is required on the maximum water content of solutions of lactitol, a method for determining the water content of solutions, and the applicability of the Method for Reducing Substances described in the Guide to specifications (FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991) as an alternative to the Luff-Schoorl Method.

Mixed carotenoids
Information is required on the composition of commercial products.

Sorbitan monolaurate
Information is required on the content of polyols of fatty acids and on an improved assay procedure.

Sulfur dioxide
Information is required on the usual levels of gaseous impurities, such as hydrogen sulfide and sulfur trioxide.

Talc
Information is required on the method for the detection of asbestos.
Annex 4

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

1. The Expert Committee was informed that the Codex Committee continued to place high priority on the elaboration of a Codex General Standard for Food Additives and that a working group had been established to develop such a standard. Its elaboration could have important implications regarding the Expert Committee's workload and priorities for future evaluations of food additives.

2. The Expert Committee was informed that the Codex Committee was elaborating "General principles for contaminants", which would include consideration of expanding the role of the Codex Committee in establishing guideline levels for naturally occurring toxicants. In this regard, it was noted that an amendment to the Codex Committee's terms of reference might be required, since naturally occurring toxicants are not considered contaminants per se.

3. The Expert Committee was advised of other discussions at the session of the Codex Committee, which included reconsideration of the specifications for gum arabic, discussion of specifications prepared at the thirty-seventh meeting of the Expert Committee, the establishment of future priorities for review, and deliberations concerning the International Numbering System, mycotoxins, and radionuclides.

4. The Expert Committee noted that, at the Twenty-third Session of the Codex Committee, several groups of substances, including phthalates, trichothecenes, polycyclic aromatic hydrocarbons, and paralytic shellfish toxins, had been placed on the priority list for future evaluation by the Expert Committee. No indication was given as to the advice that the Codex Committee wanted from the Expert Committee on these substances. Particular problems arise with naturally occurring toxicants because the toxicity data available are often incomplete and the presence of the toxicants in food is difficult to control. In addition, reducing the levels of toxicants in foods could result in changes in desirable properties, such as pest resistance, and advice to reduce the consumption of certain foods could result in nutrient imbalances.

The Expert Committee, in its thirty-seventh report (Annex 1, reference 94), recommended that substances that belong to a chemically related group should be considered as a class for the purposes of toxicological evaluation. It was likely that the classes of substances indicated above (item 4), rather than individual substances, would be placed on the priority list on the basis of this recommendation.

At its present meeting, the Committee noted that it was difficult to evaluate a large number of substances as a class, even if they were closely related chemically, because their toxicity varied. It was usually
not known whether they could be treated as a group from a toxicological point of view until the time of the evaluation itself. The Expert Committee therefore requested that Delegations to the Codex Committee indicate clearly which substances within a group are of most concern to them and the nature of the problem that has prompted them to request evaluation.
World Health Organization Technical Report Series

Recent reports:

759 (1987) Evaluation of certain food additives and contaminants
Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives (53 pages) 9.–

760 (1987) WHO Expert Committee on Biological Standardization
Thirty-seventh report (203 pages) 28.–

761 (1988) WHO Expert Committee on Drug Dependence
Twenty-fourth report (34 pages) 6.–

762 (1988) Training and education in occupational health
Report of a WHO Study Group (47 pages) 6.–

763 (1988) Evaluation of certain veterinary drug residues in food
Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives (40 pages) 6.–

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766 (1988) Strengthening ministries of health for primary health care
Report of a WHO Expert Committee (110 pages) 12.–

767 (1988) Urban vector and pest control
Eleventh report of the WHO Expert Committee on Vector Biology and Control (77 pages) 9.–

768 (1988) WHO Expert Committee on Leprosy
Sixth report (51 pages) 8.–

769 (1988) Learning together to work together for health
Report of a WHO Study Group (72 pages) 9.–

Third report of the WHO Expert Committee (63 pages) 8.–

771 (1988) WHO Expert Committee on Biological Standardization
Thirty-eighth report (221 pages) 26.–

772 (1988) Appropriate diagnostic technology in the management of cardiovascular diseases
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773 (1988) Smokeless tobacco control
Report of a WHO Study Group (81 pages) 11.–

774 (1988) Salmonellosis control; the role of animal and product hygiene
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775 (1989) WHO Expert Committee on Drug Dependence
Twenty-fifth report (48 pages) 6.–

776 (1989) Evaluation of certain food additives and contaminants
Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives (64 pages) 8.–

777 (1989) Epidemiology of work-related diseases and accidents
Tenth report of the Joint ILO/WHO Committee on Occupational Health (71 pages) 9.–

778 (1989) Health guidelines for the use of wastewater in agriculture and aquaculture
Report of a WHO Scientific Group (74 pages) 9.–

779 (1989) Health of the elderly
Report of a WHO Expert Committee (98 pages) 12.–

780 (1989) Strengthening the performance of community health workers in primary health care
Report of a WHO Study Group (46 pages) 6.–

* Prices in developing countries are 70% of those listed here.
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<td>1989</td>
<td>(1989) WHO Expert Committee on Biological Standardization Thirty-ninth report (184 pages)</td>
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<td>(1989) WHO Expert Committee on Drug Dependence Twenty-sixth report (32 pages)</td>
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<td>(1990) Pesticide application equipment for vector control Twelfth report of the WHO Expert Committee on Vector Biology and Control (58 pages)</td>
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<td>(1990) Control of the leishmaniases Report of a WHO Expert Committee (158 pages)</td>
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<td>(1990) The use of essential drugs Fourth report of the WHO Expert Committee (57 pages)</td>
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<td>(1990) Chemistry and specifications of pesticides Thirteenth report of the WHO Expert Committee on Vector Biology and Control (77 pages)</td>
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<td>(1990) WHO Expert Committee on Biological Standardization Fortieth report (221 pages)</td>
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<td>1990</td>
<td>(1990) The role of research and information systems in decision-making for the development of human resources for health Report of a WHO Study Group (54 pages)</td>
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805 (1990) Practical chemotherapy of malaria
   Report of a WHO Scientific Group (141 pages)
806 (1991) Evaluation of certain food additives and contaminants
   Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food
   Additives (56 pages)
807 (1991) Environmental health in urban development
   Report of a WHO Expert Committee (71 pages)
808 (1991) WHO Expert Committee on Drug Dependence
   Twenty-seventh report (21 pages)
809 (1991) Community involvement in health development: challenging health
   services
   Report of a WHO Study Group (60 pages)
810 (1991) Management of patients with sexually transmitted diseases
   Report of a WHO Study Group (110 pages)
811 (1991) Control of Chagas disease
   Report of a WHO Expert Committee (101 pages)
   Report of a WHO Scientific Group (80 pages)
813 (1991) Safe use of pesticides
   Fourteenth report of the WHO Expert Committee on Vector Biology
   and Control (31 pages)
814 (1991) WHO Expert Committee on Biological Standardization
   Forty-first report (84 pages)
815 (1991) Evaluation of certain veterinary drug residues in food
   Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food
   Additives (70 pages)
816 (1992) Rheumatic diseases
   Report of a WHO Scientific Group (66 pages)
817 (1992) Oral contraceptives and neoplasia
   Report of a WHO Scientific Group (52 pages)
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   Fifteenth report of the WHO Expert Committee on Vector Biology and Control
   (67 pages)
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   Report of a WHO Study Group on the Functions of Hospitals at the First
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   Report of a WHO Scientific Group (118 pages)
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   Forty-second report (89 pages)
823 (1992) WHO Expert Committee on Specifications for Pharmaceutical
   Preparations
   Thirty-second report (140 pages)
824 (1992) WHO Expert Committee on Rabies
   Eighth report (90 pages)
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   Fifth report of the WHO Expert Committee (79 pages)
826 (1992) Recent advances in oral health
   Report of a WHO Expert Committee (42 pages)
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   Report of a WHO Study Group on Primary Health Care in Urban Areas
   (42 pages)