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

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Rome, 17–26 June 2008

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

Safety evaluation of certain food additives. WHO Food Additives Series, No. 60, in press.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 5, 2008, in press.

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 IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organization 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 (JECFA) met in Rome from 17 to 26 June 2008. The meeting was opened by Dr Ezzedine Boutrif, Director, Nutrition and Consumer Protection Division of the Agriculture and Consumer Protection Department of the Food and Agriculture Organization of the United Nations (FAO), on behalf of the Directors-General of FAO and the World Health Organization (WHO). Dr Boutrif emphasized the role of the work of the Committee in providing guidance and ultimately ensuring that international food safety and quality measures are based on state-of-the-art scientific principles and provide the necessary protection of consumers' health. He also informed the Committee of the internal as well as external work that is undertaken to improve the efficiency in the achievement of the objectives of FAO and to better meet the demands of Member countries, in the areas of food security and food safety, and highlighted in particular the Declaration of the recent High Level Conference on World Food Security: the Challenges of Climate Change and Bioenergy. He emphasized that the work on provision of international scientific advice in food safety and other related topics remains an important and high priority for FAO and WHO.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the present sixty-ninth meeting had completed declaration-of-interest forms and that no conflicts had been identified. The following declared interests and potential conflicts were discussed by the Committee. Professor Andrew Renwick consulted for the International Sweeteners Association and hence did not participate in the discussions on steviol glycosides. The employer of Dr Ian Munro receives part of its revenues from consulting on the safety assessment of food additives. The company, but not Dr Munro himself, prepared submissions regarding the assessments of steviol glycosides. Dr Paul Kuznesof consulted for Tate & Lyle to gather publicly available information on steviol glycosides, but this activity was not regarded as a conflict of interest. Professor Ron Walker consulted for one of the producing companies on calcium lignosulfonate and hence did not participate in the discussion.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been 68 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of recommendations made at previous meetings of the Committee and on request of the Codex Alimentarius Commission and Member States.

The tasks before the Committee were:

- to elaborate further principles for evaluating the safety of food additives, in particular additional considerations on the assessment of dietary exposure to flavouring agents (section 2);
- to undertake toxicological evaluations of certain food additives (sections 3 and 4 and Annex 2);
- to review and prepare specifications for certain food additives (sections 3 and 4 and Annex 2).

2.1 Modification of the agenda

When discussing the compounds lauric arginate ethyl ester, ligninsulfonate and phospholipase C from *Pichia pastoris*, which were on the agenda for evaluation for the first time, the Committee considered the names inappropriate and decided to rename them, respectively, ethyl lauroyl arginate, calcium lignosulfonate (40–65) and phospholipase C expressed in *Pichia pastoris*. In addition, the flavouring agent (No. 1846) 3-hexenyl 2- oxopropionate was renamed (*Z*)-3-hexenyl 2-oxopropionate, as the substance evaluated was the *Z*-isomer.

The re-evaluation of the safety of mineral oils (low and medium viscosity), classes II and III, was deferred to a future meeting. The Committee received information from the sponsor that relevant studies are being undertaken and agreed to maintain the temporary acceptable daily intake (ADI) until the end of 2009, awaiting additional data to be submitted.

The food additives canthaxanthin; chlorophyllin copper complexes, sodium and potassium salts; Fast Green FCF; iron oxides; and isomalt were added to the agenda for revision of specifications.

2.2 Report from the Fortieth Session of the Codex Committee on Food Additives (CCFA) and the Second Session of the Codex Committee on Contaminants in Food (CCCF)

The Chairman of the Codex Committee on Food Additives (CCFA), Dr Junshi Chen, informed the Committee about the principal achievements and output of the Fortieth Session of CCFA. CCFA proposed about 320 provisions for food additives for adoption by the Codex Alimentarius Commission. Sixteen JECFA specifications for food additives and 172 specifications for flavouring agents were also proposed for adoption as Codex specifications, and three were proposed to be revoked. CCFA agreed on a revised guideline for the use of flavourings for adoption at step 8 and step 5/8 of the Codex procedure, following the finalization of the elaborations on how to address naturally occurring flavouring complexes. Such substances may in the future be subject to specific risk management procedures based on evaluations by the Committee. CCFA also proposed to start new work on a Codex guideline on the use of processing aids. Dr Chen also informed the Committee that an answer had been provided to the Codex Committee on Nutrition and Foods for Special Dietary Uses on the question related to the non-applicability of acceptable daily intakes (ADIs) established by the Committee for infants aged less than 12 weeks in the absence of specific data, based on previous considerations and decisions by the Committee. Finally, CCFA agreed on a list of food additives proposed for evaluation by JECFA at future meetings.

The Secretariat summarized key discussions of the Second Session of the Codex Committee on Contaminants in Food (CCCF), which was based on assessments provided by JECFA. Maximum limits were proposed for 3-monochloropropane-1,2-diol (3-MCPD) in liquid condiments containing acid-hydrolysed vegetable proteins (excluding naturally fermented soya sauce); ochratoxin A in raw wheat, barley and rye; and total aflatoxins in the tree nuts almonds, hazelnuts and pistachios (nuts ready to eat and nuts for further processing) for adoption at step 8 of the Codex procedure. CCCF agreed on a priority list of substances to be evaluated by JECFA and also on the need for development of discussion papers on occurrence and identification of hazards related to other contaminants for which concern had been expressed by delegations attending the Second Session of CCCF.

2.3 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives, 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 the principles elaborated at subsequent meetings of the Committee (Annex 1, references *77*, *83*, *88*, *94*, *101*, *107*, *116*, *122*, *131*, *137*, *143*, *149*, *152*, *154*, *160*, *166*, *173*, *176*, *178*, *184* and *187*), including the present one. WHO Environmental Health Criteria, No. 70, contains the most important observations, comments and recommendations made, up to the time of its publication, by the Committee and associated bodies in their reports on the safety assessment of food additives and contaminants.

2.4 The safety evaluation of flavouring agents

2.4.1 *Dietary exposure assessment of flavouring agents: Incorporation of the single portion exposure technique (SPET) into the Procedure for the Safety Evaluation of Flavouring Agents*

Introduction

JECFA employs the maximized survey-derived intake (MSDI) method as a measure of the dietary exposure to flavouring agents for use in the Procedure for the Safety Evaluation of Flavouring Agents (the Procedure). The MSDI provides a per capita estimate of the dietary exposure to a flavouring agent that is compared with the relevant threshold of toxicological concern (TTC) for each structural class in a decision tree approach according to the Procedure. The MSDI is based on the reported amount of the flavouring agent introduced into the food supply per year in specific regions, currently Europe, the United States of America (USA) and Japan, corrected for under-reporting, and assuming that 10% of the relevant population would consume foods containing the flavouring agent.

The Committee considered issues related to dietary exposure to flavouring agents at its forty-fourth, forty-sixth, forty-ninth, fifty-fifth, sixty-third, sixty-fifth, sixty-seventh and sixty-eighth meetings (Annex 1, references *116*, *122*, *131*, *149*, *173*, *178*, *184* and *187*). The main concern expressed by the Committee was that the MSDI method may significantly underestimate dietary exposure to some flavouring agents. This could be the case for flavouring agents consumed by less than 10% of the population, especially where they might be used in a few food categories, and for flavouring agents with an uneven distribution of dietary exposure among consumers. The uneven distribution might be due to a combination of factors, including different use

levels across and within food categories, restriction to use in a few foods or food categories and different levels of consumption for different foods.

The single portion exposure technique (SPET) was developed by the Committee at its sixty-seventh meeting (Annex 1, reference *184*) to account for presumed patterns of consumer behaviour with respect to food consumption and the possible uneven distribution of dietary exposure for consumers of foods containing flavouring agents. The SPET provides an estimate of dietary exposure for an individual who consumes a specific food product containing the flavouring agent every day. The SPET combines an average (or usual) added use level with a standard portion size for a food category. Among all the food categories with a reported use level, the dietary exposure from the single food category leading to the highest dietary exposure from one portion is taken as the SPET estimate. The standard portion does not reflect high levels of food consumption reported in national dietary surveys. It was intended that the higher value of the two dietary exposure estimates (MSDI or SPET) would be used within the Procedure.

At its sixty-eighth meeting and its present meeting, the Committee performed a number of SPET and MSDI calculations with the aim of:

- determining whether a set of criteria could be identified for future selection of flavouring agents for which the MSDI could underestimate dietary exposure. In these cases, extra information on added use levels recommended by the industry would be required to calculate a SPET estimate;
- evaluating the possible impact of using both the MSDI and SPET estimates of dietary exposure in the Procedure for different flavour groups.

Investigation to develop criteria for the identification of flavouring agents requiring additional consideration

At its sixty-eighth meeting, the Committee calculated SPET estimates for 57 flavouring agents based on use levels provided by the International Organization of the Flavor Industry (IOFI),[1] 44 with low production volumes (<10 kg/year) and 13 with intermediate to high production volumes (production volumes corresponding to an amount that was greater than one third of the relevant TTC). These flavouring agents were selected from all structural classes and eight different groups. For 4 of the 57 flavouring agents selected, the MSDI was greater than the corresponding SPET estimate. Although for the remaining 53 flavouring agents the SPET estimate was greater than the corresponding MSDI, different steps through the Procedure would have been

[1] IOFI collated data on added use levels from the European Flavour and Fragrance Association (EFFA), the Flavor and Extract Manufacturers Association of the USA (FEMA) and the Japan Flavor & Fragrance Materials Association (JFFMA) and submitted these data on behalf of the three organizations.

required in only two cases where the SPET estimate exceeded the relevant TTC. The Committee concluded that, using this small group of flavours for the analysis, it was not possible to develop any selection criteria (based on production volume, structural class or flavour group) to identify cases where the MSDI would have underestimated dietary exposure and different steps through the Procedure would have been required if the SPET estimate were to be used in the Procedure. Consequently, for the present meeting of the Committee, additional data on use levels for another set of flavouring agents with intermediate to high volumes of production were requested from and provided by IOFI to extend the analysis.

Analysis of data for 40 flavouring agents considered at the present meeting

IOFI data were made available to calculate SPET estimates for 40 flavouring agents from 15 different flavour groups with intermediate to high production volumes. Of these, 28 were in structural class I, 6 in class II and 6 in class III. For class I flavouring agents, none of the SPET estimates exceeded the TTC, whereas the MSDI exceeded the TTC in one case. For class II flavouring agents, one SPET estimate exceeded the TTC, whereas no MSDI estimates exceeded the TTC. For class III flavouring agents, all six SPET estimates exceeded the TTC, whereas two of the MSDI estimates exceeded the TTC. Cases where the SPET estimate exceeded the MSDI and exceeded the TTC occurred in this group of flavouring agents across different production volumes, structural classes and flavour groups, a similar finding to that for the 57 flavouring agents considered at the sixty-eighth meeting.

Analysis of a larger data set of flavouring agents

Because the analyses of flavouring agents considered at the sixty-eighth meeting and the present meeting were inconclusive, the Committee collected use level data from other sources to determine whether suitable criteria for predicting when the MSDI might underestimate dietary exposure could be developed based on a larger group of flavouring agents. Additionally, the likelihood that the SPET estimate would exceed the relevant TTC when the MSDI did not was examined. Overall, SPET estimates for 549 flavouring agents were calculated, based on use levels derived from three main data sets:

- for 225 flavouring agents: recent and refined[1] use level data provided by IOFI to the Committee or to the European Commission (Directorate

[1] In this context, “refined” means that the information is derived from use levels in specific foods or food types, rather than broad food categories (e.g. “fruit-flavoured yogurt” as opposed to “dairy products”).

General for Health and Consumer Affairs [DG SANCO]) in 2007 and 2008;

- for 198 flavouring agents: refined[2] use level data collected in an industry survey (National Academy of Sciences/National Research Council [NAS/NRC]) conducted in the USA in 1977;
- for 268 flavouring agents: use levels proposed by industry for flavouring agents registered as FEMA Generally Recognized as Safe (GRAS),[2] published between 1965 and 2007.

Some flavouring agents were assessed using more than one source of use levels, resulting in a total of 691 SPET estimates.

Some of the portion sizes used in the SPET calculations were updated at the present meeting based on reported food consumption levels, including the addition of new portion sizes (Table 1).

Table 1
Updated portion sizes to be used for the calculation of SPET estimates

Food categorization system for the Codex General Standard for Food Additives (GSFA) (see http://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf)	Standard portion (g) (sixty-seventh meeting of Committee)	Revised standard portion (g) (present meeting of Committee)	Notes
01.0 Dairy products and analogues, excluding products of category 02.0			
01.1 Milk and dairy-based drinks	200	200 (30*)	
01.2 Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks)	200	200 (30*)	
01.3 Condensed milk and analogues	NA	70	Differs from United States standard portion, which refers only to milk added to coffee, tea, etc.
01.4 Cream (plain) and the like	NA	15	

[2] GRAS is a regulatory concept specific to the United States Federal Food, Drug, and Cosmetic Act. Any substance added to food requires a food additive regulation for its use, unless its intended use is GRAS. Food ingredients whose use is GRAS are not required by law to receive Food and Drug Administration (FDA) approval before marketing. FEMA has been publishing lists of flavouring substances, and associated use levels at or below which it has deemed their use to be GRAS, for more than 30 years.

01.5 Milk powder and cream powder and powder analogues (plain)	NA	30*	Differs from United States standard portion, which refers only to milk added to coffee, tea, etc.
01.8 Whey and whey products, excluding whey cheeses	NA	200 (30*)	
04.0 Fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes and aloe vera), seaweeds, and nuts and seeds			
04.1 Fruit			
04.1.1 Fresh fruit	NA	140	
04.1.2.5 Jams, jellies, marmalades	NA	30	
04.2 Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds			
04.2.2.5 Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter)	NA	30	For nut and similar spreads
06.0 Cereals and cereal products derived from cereal grains, roots and tubers, and pulses and legumes, excluding bakery wares of food category 07.0			
06.1 Whole, broken or flaked grain, including rice	NA	200 (70 raw)	
06.2 Flours and starches (including soya bean powder)	NA	30	
06.5 Cereal and starch-based desserts (e.g. rice pudding, tapioca pudding)	200	200 (30*)	For pudding powder
08.0 Meat and meat products, including poultry and game			
08.1 Fresh meat, poultry and game	NA	200	
08.4 Edible casings (e.g. sausage casings)	NA	1	
09.0 Fish and fish products, including molluscs, crustaceans and echinoderms			
09.1 Fresh fish and fish products, including molluscs, crustaceans and echinoderms			
09.1.1 Fresh fish	NA	200	

09.1.2 Fresh molluscs, crustaceans and echinoderms	NA	200
09.2 Processed fish and fish products, including molluscs, crustaceans and echinoderms	100	100
09.3 Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms	100	100
09.4 Fully preserved, including canned or fermented, fish and fish products, including molluscs, crustaceans and echinoderms	100	100
10.0 Eggs and egg products		
10.1 Fresh eggs	NA	100
11.0 Sweeteners, including honey		
11.6 Table-top sweeteners, including those containing high-intensity sweeteners	15	1
12.0 Salts, spices, soups, sauces, salads, protein products (including soya bean protein products) and fermented soya bean products		
12.1 Salt and salt substitutes	NA	1
12.5 Soups and broths	200	200 (30*)
12.8 Yeast and like products	NA	1
12.9 Protein products	15	15
13.0 Foodstuffs intended for particular nutritional uses		
13.1 Infant formulae, follow-on formulae and formulae for special medical purposes for infants	NA	1000
13.2 Complementary foods for infants and young children	NA	50
13.3 Dietetic foods intended for special medical purposes (excluding food products of category 13.1)	NA	200 (30*)
13.4 Dietetic formulae for slimming purposes and weight reduction	NA	200 (30*)
13.5 Dietetic foods (e.g. supplementary foods for dietary use) excluding products of food categories 13.1–13.4 and 13.6	NA	200 (30*)
14.0 Beverages, excluding dairy products		
14.1 Non-alcoholic (“soft”) beverages	300	300 (12 for coffee or 30 for drink mix powders*)
14.2 Alcoholic beverages, including alcohol-free and low-alcoholic counterparts		

14.2.5 Mead	NA	150	The portion size is derived from that of Grape wines (14.2.3)
16.0 Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15	NA	300	Reported uses

NA, not available
* In parentheses, the amount is applicable for powder.

In nearly all cases (92%), the SPET estimate was greater than the MSDI, and it was more likely that the SPET estimate was greater than the TTC of the relevant structural class than the corresponding MSDI. The SPET estimate was most frequently greater than the TTC in class III, but this also occurred in classes I and II (see Table 2).

Table 2
Comparison of SPET and MSDI with TTC for flavouring agents in structural classes I, II and III

	Source of use level data		
	IOFI 2007–2008 (*n* = 225)	NAS/NRC 1977 (*n* = 198)	FEMA GRAS 1965–2007 (*n* = 268)
Class I, SPET > TTC	1/70 (1%)	38/121 (31%)	25/111 (23%)
Class II, SPET > TTC	1/12 (8%)	13/58 (22%)	32/62 (52%)
Class III, SPET > TTC	86/143 (60%)	12/19 (63%)	77/95 (81%)
Total, SPET > TTC	**88/225 (39%)**	**63/198 (32%)**	**134/268 (50%)**
Class I, MSDI > TTC	2/70 (3%)	5/121 (4%)	1/111 (1%)
Class II , MSDI > TTC	0/12 (0%)	4/58 (7%)	1/62 (2%)
Class III, MSDI > TTC	12/143 (8%)	1/19 (5%)	12/95 (13%)
Total, MSDI > TTC	**14/225 (6%)**	**10/198 (5%)**	**14/268 (5%)**

Note: Some flavouring agents were assessed using more than one source of use levels.

The Committee considered the use of FEMA GRAS use levels to be less desirable than that of the more specific use levels provided by IOFI, as FEMA GRAS values are projected and probably overestimate actual added use levels. IOFI provided high-quality use level data from recent surveys and informed the Committee that, with very few exceptions, there is a strong agreement between recent and older use level surveys and that comparison of these surveys supports the conclusion that use levels for flavouring agents

with similar flavouring effect are generally similar and have not changed significantly over time.

For the flavouring agents with IOFI use level data only, the differences between the two dietary exposure estimates were examined. The Committee considered that it would be inappropriate to use the SPET estimates based on NAS/NRC data from 1977 or FEMA GRAS levels for this purpose.

Overall, for the group of 225 flavouring agents with IOFI use level data, 50% had a SPET estimate that was less than 2 orders of magnitude higher than the MSDI (median ratio of SPET to MSDI was 85). Twenty-one flavouring agents had an MSDI that was higher than the SPET estimate by up to 2 orders of magnitude. For the remaining 204 flavouring agents, the SPET estimate was higher than the MSDI. Of these, 24 had SPET estimates that were 4–6 orders of magnitude higher than the MSDI.

From the analysis of the MSDI and SPET estimates for the 549 flavouring agents, the Committee concluded that it was not possible to develop criteria, based on production volume, structural class or flavour group, to predict when the MSDI might underestimate dietary exposure and when the SPET estimate, but not the MSDI, was likely to exceed the TTC.

Consideration of the incorporation of the SPET estimate into the Procedure

At its present meeting, the Committee considered the consequences of incorporating the SPET estimate into the Procedure, using two flavour groups as an example. One group was evaluated on the A-side of the Procedure (six hydroxy- and alkoxy-substituted benzyl derivatives; section 4.1.7), and one group on the B-side (14 miscellaneous nitrogen-containing substances; section 4.1.8). In four cases, IOFI use level data were available. For the other 16 flavouring agents, FEMA GRAS levels were used for the SPET estimate for the purposes of this exercise only, as these were the only use levels available.

For these two groups of flavouring agents, the food categories responsible for the highest dietary exposure in one standard portion were beverages, either alcoholic or non-alcoholic (for nine flavouring agents), processed fruit (two cases), processed vegetables (one case), meat products (two cases), cereals and cereal products such as baked goods (four cases), condiments (one case) and milk and dairy-based drinks (one case).

*Hydroxy- and alkoxy-substituted benzyl derivatives***.** In applying the Procedure for the Safety Evaluation of Flavouring Agents using the MSDI for the six flavouring agents in the hydroxy- and alkoxy-substituted benzyl derivatives group of flavouring agents, the Committee assigned five flavouring agents (Nos 1878–1880, 1882 and 1883) to structural class I and the

remaining flavouring agent (No. 1881) to structural class III (*2*). The evaluation of all agents in this group proceeded via the A-side of the Procedure. According to the Procedure using the MSDI, the safety of these six flavouring agents raised no concern, because the dietary exposure was below the relevant TTC.

Incorporation of the SPET estimate into the Procedure would have resulted in different steps through the Procedure for three of the six flavouring agents. SPET estimates based on IOFI use levels were available for only one of the flavouring agents in this group (No. 1882). The estimated dietary exposure to sodium 4-methoxybenzoyloxyacetate (No. 1880) and 4- methoxybenzoyloxyacetic acid (No. 1883) exceeded the TTC for structural class I (1800 μg/day) using the SPET estimate. Similarly, the dietary exposure to divanillin (No. 1881) exceeded the TTC for structural class III (90 μg/day).

Miscellaneous nitrogen-containing substances. In applying the Procedure for the Safety Evaluation of Flavouring Agents using the MSDI for the 14 flavouring agents in the group of miscellaneous nitrogen-containing substances, the Committee assigned 12 (Nos 1884–1890, 1892–1894, 1896 and 1897) to structural class II and the remaining 2 (Nos 1891 and 1895) to structural class III (*2*). None of the flavouring agents in this group could be predicted to be metabolized to innocuous products. The evaluation of these 14 flavouring agents therefore proceeded via the B-side of the Procedure. According to the Procedure using the MSDI, the safety of these 14 flavouring agents raised no concern.

Incorporation of the SPET estimate into the Procedure would have resulted in different steps through the Procedure for 2 of the 14 flavouring agents (Nos 1894 and 1895), as they would not have progressed to step B4. SPET estimates based on IOFI use levels were available for only three flavouring agents in this group (Nos 1889, 1893 and 1894).

Conclusion. The results for these two flavour groups indicated that the incorporation of the SPET estimate into the Procedure for flavouring agents going through the A-side of the Procedure will more often require appropriate toxicity data on these flavouring agents or on closely related substances to complete the safety evaluation at step A5. For flavouring agents going through the B-side of the Procedure, additional toxicological data will more often be required for those flavouring agents that do not progress to step B4. In all these cases, additional data would need to be included in the submission for the flavouring agents. IOFI use level data would need to be submitted in the data package for all flavouring agents going through either side of the Procedure to enable SPET estimates to be made.

Combined dietary exposure

The SPET estimate for a flavouring agent represents the dietary exposure for a daily consumer of a standard portion of food containing the substance. The combination of SPET estimates for related flavouring agents could greatly overestimate dietary exposure. The Committee therefore considered that the estimate of combined dietary exposure in the Procedure should continue to be based on the MSDI estimates, as outlined in the report of the sixty-eighth meeting.

Conclusion

The Committee noted that MSDI and SPET estimates of dietary exposure provide different and complementary information. Use of the SPET estimate addresses previous concerns expressed by the Committee about the dietary exposure methodology used in the Procedure, because the SPET estimates take account of the possible uneven distribution of dietary exposures to a flavouring agent for consumers of foods containing that substance. The higher value of the two dietary exposure estimates (MSDI or SPET) should be used within the Procedure.

As it was not possible to elaborate criteria to identify the flavouring agents for which the MSDI underestimated dietary exposure and SPET estimates should be used, the Committee concluded that it was necessary to incorporate SPET estimates into the Procedure for all flavouring agents considered at future meetings of the Committee. The Committee agreed that it would not be necessary to re-evaluate flavouring agents that have already been assessed using the Procedure.

To enable a safety evaluation using the Procedure to be undertaken, the Committee requested that added use level data be provided for each flavouring agent in a timely fashion before the meeting, in addition to up-to-date data on production volumes, as part of the data package for the safety evaluation. The Committee will not perform a safety evaluation in the absence of such data.

2.4.2 *Considerations on the thresholds of toxicological concern used in the Procedure*

The Committee received prepublication copies of a paper (*3*) on the use of TTCs in the safety evaluation of flavouring agents and in other risk assessment applications. The TTC values used in the Procedure for the Safety Evaluation of Flavouring Agents for structural classes I, II and III (1800, 540 and 90 μg/person per day, respectively) were derived from analyses of toxicity data for a wide range of chemicals and not just flavouring agents. The

TTC values were calculated by dividing the 5th percentiles of the distributions of no-observed-adverse-effect levels (NOAELs) for each structural class by a 100-fold uncertainty factor and multiplying by an average body weight (bw) of 60 kg. NOAELs of 3.0, 0.91 and 0.15 mg/kg bw per day had been derived from toxicity data on 137, 28 and 448 compounds in structural classes I, II and III, respectively.

The distribution of NOAELs for class III compounds was influenced markedly by the presence of neurotoxic organophosphate and organohalogen pesticides in the database used. The recent publication (*3*) showed that exclusion of compounds with these chemical characteristics, which are not representative of the structures of flavouring agents, would result in a 5th percentile of the distribution of NOAELs for structural class III of about 1.0 mg/kg bw per day, giving a revised TTC value of about 600 μg/person per day, which is similar to that for structural class II.

The Committee is aware that there are various activities currently under way to update and revise the Cramer decision tree (*2*), which is used to determine the structural class, and also to update the toxicology database used to establish the TTC values. There is widespread interest in developing TTC values appropriate to specific applications, such as flavouring agents, certain food additives and residues of pesticides and veterinary drugs in food. The Committee considered that this subject should be discussed in depth at a future meeting.

2.5 Food additive specifications

2.5.1 *Withdrawal of specifications*

2.5.1.1 *Carbohydrase from* Aspergillus niger *varieties*

The Committee reviewed the tentative specifications for carbohydrase from *Aspergillus niger* varieties that had been prepared at its fifteenth meeting (Annex 1, reference *26*) and for which an ADI "not specified" was established at its thirty-fifth meeting (Annex 1, reference *88*). The call for data for the sixty-ninth meeting requested information to revise the existing tentative specifications, stating that the specifications would be withdrawn if no information was forthcoming.

The tentative specifications for carbohydrase include α-amylase, pectinase, cellulase, glucoamylase and β-galactosidase (lactase). The functional uses listed in the specifications are diverse and imply that these enzymes are used in food processing as separate enzyme preparations rather than as a mixture of enzymes. Moreover, carbohydrase is not listed as a commercial enzyme

by the enzyme industry associations, whereas all individual enzymes included in the tentative specifications are listed as commercial products.

As no information supporting the tentative specifications was received, the Committee withdrew the ADI and the tentative specifications.

2.5.1.2 *Estragole*

The tentative specifications for estragole used as a food additive that were prepared by the Committee at its twenty-sixth meeting, published in FAO Food and Nutrition Paper No. 25 (Annex 1, reference *61*) and republished in the Combined Compendium for Food Additive Specifications (Annex 1, reference *180*), were withdrawn, as no uses of estragole other than as a flavouring agent were identified.

2.5.2 *Method for determination of nickel in polyols*

When reviewing the specifications for isomalt, the Committee recognized that the method for determination of nickel in polyols described in Volume 4 of the Combined Compendium for Food Additive Specifications (Annex 1, reference *180*) was incomplete. The method was revised and will be published in the Compendium of Food Additive Specifications, FAO JECFA Monographs 5 (Annex 1, reference *192*).

2.6 Relationship between the ADI and specifications

The Committee has repeatedly stressed the important relationship between the ADI and specifications for material(s) to which the ADI applies. As indicated in WHO Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference *76*):

> Specifications are a necessary product of Committee evaluations, the purposes of which are 3-fold:
>
> (a) to identify the substance that has been biologically tested;
>
> (b) to ensure that the substance is of the quality required for safe use in food; and
>
> (c) to reflect and encourage good manufacturing practice.

At its fifteenth meeting (Annex 1, reference *26*), the Committee stated that:

> JECFA specifications in their entirety describe substances of food-grade quality, and as such, they are directly related to toxicological evaluations and to good manufacturing practice. However, though specifications may include criteria that are important for commercial

users of additives, they do not include requirements that are of interest only to commercial users.

Furthermore, when considering implications of extending existing ADIs to substances obtained from different sources and/or by different manufacturing processes, the Committee, at its sixth-eighth meeting (Annex 1, reference *187*), noted that "the guiding principle in the safety evaluation has been that the material tested toxicologically is representative of the material of commerce".

At the current meeting, the Committee emphasized the importance of this relationship between specifications and the ADI. It noted that changes in specifications may raise questions concerning the relationship between the material tested toxicologically, on which the safety assessment is based, and the material of commerce.

The Committee recommends that when proposals are made to include or revise limits for impurities or when compositional changes occur that lead to a need for revision of the specifications, the consequences for the safety assessment of the substance need to be considered.

Considerations on potentially necessary data requirements and re-evaluation of the safety of the specified material need to be taken into account by the JECFA Secretariat and by CCFA when requesting changes to existing specifications.

3. Specific food additives (other than flavouring agents)

The Committee evaluated five food additives, including the group of phytosterols, phytostanols and their esters, for the first time and re-evaluated a number of others. Information on the safety evaluations and on specifications is summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 Safety evaluations

3.1.1 *Asparaginase from* Aspergillus niger *expressed in* A. niger

Explanation

At the request of CCFA at its Thirty-ninth Session (*4*), the Committee evaluated a preparation containing the enzyme asparaginase (L-asparagine amidohydrolase; Enzyme Commission [EC] No. 3.5.1.1) derived from a genetically modified strain of *Aspergillus niger*. The Committee had previously evaluated asparaginase from a genetically modified strain of *Aspergillus oryzae* at its sixty-eighth meeting (Annex 1, reference *187*). Asparaginase catalyses the hydrolysis of L-asparagine to L-aspartic acid and ammonia. The enzyme is to be added during the manufacture of bread and other cereal-based products and baked and fried potato-based products, where the enzyme is added before heat treatment of these products with the intention of reducing the formation of acrylamide.

Genetic modification

Asparaginase is manufactured by pure culture fermentation of a genetically modified strain of *A. niger* that contains multiple copies of the asparaginase gene derived from *A. niger*, which were inserted into predetermined loci in the *A. niger* genome. *Aspergillus niger* is a filamentous fungus that commonly occurs in the environment and is considered to be non-pathogenic. The asparaginase production strain was constructed by transformation of the *A. niger* host strain DS 51563 with deoxyribonucleic acid (DNA) fragments derived from two plasmids, one containing the asparaginase gene from *A.*

niger and the other containing the acetamidase gene from *A. nidulans.* The acetamidase gene was used as a selectable marker to identify transformants and was subsequently removed from the production strain. As a result, the asparaginase production strain contains multiple copies of the *A. niger* asparaginase gene but no other heterologous genes. The asparaginase production strain was evaluated for its potential to produce toxic secondary metabolites, including ochratoxins. There was no indication of the formation of toxic secondary metabolites under the fermentation conditions used in the production of asparaginase.

Chemical and technical considerations

Asparaginase is secreted to the fermentation broth and is subsequently purified and concentrated. The enzyme concentrate is formulated and standardized into either a liquid or a granulated preparation using appropriate food-grade substances. The asparaginase preparation complies with the General Specifications and Considerations for Enzyme Preparations Used in Food Processing prepared by the Committee at its sixty-seventh meeting (Annex 1, reference *184*) and does not contain viable cells of the production organism. The total organic solids (TOS) content of the asparaginase preparation may vary from 6% to 10%.

Since the asparaginase preparation is added to food before heat treatment to reduce the availability of L-asparagine for acrylamide formation, it will subsequently be inactivated by denaturation during the heating/baking step. The TOS residues in the final food (including denatured asparaginase) may range from 0.14 to 428 mg/kg of the final food. The effectiveness of the asparaginase enzyme preparation in reducing acrylamide formation was not evaluated by the Committee.

Toxicological data

Toxicological studies were performed with the asparaginase enzyme using a representative batch (APE0604), which was produced according to the procedure used for commercial production. The liquid enzyme concentrate was spray-dried to produce the final, non-formulated test substance, with an average activity of 34 552 asparaginase units (ASPU)/g and a TOS value of 89.7% before addition to the feed. In a 13-week study of general toxicity and a study of developmental toxicity in rats, no significant treatment-related effects were seen when this material was administered in the feed at concentrations of up to 1.8% by weight (w/w). Therefore, 1038 mg TOS/kg bw per day, the highest dose tested, was taken to be the no-observed-effect level (NOEL). Asparaginase was not mutagenic in an assay for mutagenicity in

bacteria in vitro and was not clastogenic in an assay for chromosomal aberration in mammalian cells in vitro.

Asparaginase was evaluated for potential allergenicity according to the bioinformatics criteria recommended by FAO/WHO (*5*). The amino acid sequence of asparaginase was compared with the amino acid sequences of known allergens. No sequence homology that would suggest that asparaginase is an allergen was identified.

Assessment of dietary exposure

An estimate of dietary exposure was made by the Committee based on the 13 Consumption Cluster Diets of the Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) categorization[1] and on the Concise European Food Consumption Database for the adult population (age 16–64 years). The European database compiles mean and high percentiles of individual food consumption for 15 broad food categories from the majority of European countries ($n = 17$). The GEMS/Food cluster diets report per capita daily consumption of food commodities. In these estimates, reported consumption data have been combined with the maximum use levels recommended. This corresponds to 23 mg TOS/kg food for cereal-based products and 428 mg TOS/kg food for potato-based products. For the GEMS/Food data, the food categories used in the calculation were cereals and root and tuber commodities. For the European database, the food categories used were cereals and cereal products and starchy roots or potato products.

The potential mean dietary exposure to asparaginase from *A. niger* based on international and national conservative estimates for the adult population, assuming a body weight of 60 kg, range from 0.5 to 3.7 mg TOS/kg bw per day (0.5–1.7 mg TOS/kg bw per day for Europe and 0.8–3.7 mg TOS/kg bw per day based on GEMS/Food cluster diets) and from 1.1 to 4.1 mg TOS/kg bw per day for high-percentile consumers (95th percentile) in Europe.

The Committee noted that these results were conservative because they assume the consumption of foods from two (of the 15) broad food categories, both of which contained asparaginase at the highest reported use levels.

Evaluation

Comparing the most conservative estimate of exposure (i.e. 4.1 mg TOS/kg bw per day) with the NOEL of 1038 mg TOS/kg bw per day from the 13-week study of oral toxicity, the margin of exposure is about 250. The

[1] For more details on the GEMS/Food Consumption Cluster Diets, see http://www.who.int/foodsafety/chem/gems/en/index1.html.

Committee allocated an ADI "not specified" for asparaginase from *A. niger* expressed in *A. niger* used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

A Chemical and Technical Assessment and new specifications were prepared.

3.1.2 *Calcium lignosulfonate (40–65)*

Explanation

This substance, under the name "ligninsulfonate", was placed on the agenda of the present meeting at the request of CCFA at its Thirty-ninth Session (*4*) for assessment of safety, specifications and dietary exposure. The Committee received information only on calcium lignosulfonate and decided to refer to the specified material as "calcium lignosulfonate (40– 65)" to distinguish it from other calcium lignosulfonates on the market. The number included in the name of the additive reflects the weight-average molecular weight range (40 000– 65 000) specified in the specifications monograph developed by the Committee at its present meeting. Calcium lignosulfonate (40–65) is intended for use as a carrier of encapsulated food ingredients. It has not been evaluated previously by the Committee.

Chemical and technical considerations

Calcium lignosulfonate (40–65) is an amorphous light yellow-brown to brown powder obtained from the sulfite pulping of soft wood; it is derived from lignin, the second largest component of wood. The additive is soluble in water, but not in common organic solvents. Owing to its water solubility, calcium lignosulfonate (40–65) can serve as a protective colloid for formulations of fat-soluble vitamins, carotenoids and food colours.

Lignosulfonates are commercially available as sodium and calcium salts and have been used by industry in a wide variety of applications. The usefulness of commercial products containing lignosulfonates comes from their dispersing, binding, complexing and emulsifying properties. The additive calcium lignosulfonate (40–65) evaluated at the present meeting presents a higher degree of lignin polymerization and a lower content of sugars than do other calcium lignosulfonates on the market. The lignin framework of the additive is a sulfonated random polymer of three aromatic alcohols (phenylpropane monomers): coniferyl alcohol, *p*-coumaryl alcohol and sinapyl alcohol, of which coniferyl alcohol is the principal unit. The additive exhibits a weight-average molecular weight in the range of 40 000–65 000, with more than 90%

of the polymer constituents having molecular weights ranging from 1000 to 250 000.

Calcium lignosulfonate (40–65) is intended for use as a carrier for the production of encapsulated fat-soluble vitamins (A, D, E and K) and carotenoids (e.g. β-carotene, β-apo-8′-carotenal, zeaxanthin, canthaxanthin, lutein and lycopene) to facilitate their introduction into water-based foods. It has an adequate emulsifying and film-forming effect and viscosity that ensure the formation of droplets of appropriate size in the final step of the encapsulation process. Potential applications of the encapsulated ingredients include their uses in, for example, fruit-based beverages, vitamin drinks, dairy products and hard candies. The additive can be used in much the same way as other water-soluble matrix materials, such as gelatins, gum arabic, soya protein hydrolysates and modified starches.

The Committee reviewed data on stability studies with the additive itself, with the additive in carotenoid preparations and with a β-carotene/additive-containing product used in a non-pasteurized, non-carbonated soft drink. The Committee concluded that the stability of the additive is adequate for the intended uses.

Toxicological data

Studies with tritiated calcium lignosulfonate (40–65) in rats indicated that only limited absorption occurs after oral exposure. Owing to the constant formation of tritiated water from the product, most (98.5%) of the radioactivity in blood, tissues and urine co-eluted with tritiated water, indicating that only about 1% was present in higher molecular weight fractions of the purified material used for dosing.

The toxicity of calcium lignosulfonate (40–65) has been studied in 28-day and 90-day studies of oral toxicity in which calcium lignosulfonate (40–65) was incorporated into the diet. In the 28-day study of toxicity, groups of male and female Wistar rats were given diets providing calcium lignosulfonate (40–65) at a target daily dose of 0, 500, 1500 or 4000 mg/kg bw. The study was carried out in accordance with Organisation for Economic Co-operation and Development (OECD) guidelines and involved complete pathological examination of all major organs. With the exception of chronic inflammation of the rectum in males at the highest dose, but not at the lowest or intermediate dose, no adverse effects were observed. The NOAEL was equal to 1300 mg/kg bw per day for males and 1350 mg/kg bw per day for females on the basis of the inflammatory response in the rectum.

In a 90-day study that complied with Good Laboratory Practice (GLP) and with OECD guidelines, groups of male and female Wistar rats were given

diets providing calcium lignosulfonate (40–65) at a target dose of 0, 500, 1000 or 2000 mg/kg bw per day. This study involved complete pathological examination of all organs and tissues. No adverse clinical or organ weight changes were reported. A functional observational battery provided no evidence of adverse effects, and the results of a test for primary immune response were normal. In this study, no histopathological changes were noted in the rectum, but there was a dose-related increase in the incidence of histiocytosis of the mesenteric lymph nodes in male and female rats. The magnitude of this effect also increased with dose. The incidence and magnitude of this effect showed minimal regression in a 28-day recovery study conducted in satellite groups of rats. There was no evidence of histiocytosis in other lymphoreticular tissues. There was also an increase in the incidence of tubular vacuolation of the kidney, but this was not accompanied by a degenerative change and therefore was not considered to be an adverse effect.

The finding of histiocytosis in the mesenteric lymph nodes of rats treated with calcium lignosulfonate (40–65) has also been observed with other high molecular weight, poorly absorbed materials, such as petroleum-derived mineral oils and waxes and copovidone (a copolymer of vinylpyrrolidone and vinyl acetate). Similar effects have also been observed with polypentosan sulfate. Histiocytosis appears to be related to an attempt by the histiocytes of the mesenteric lymph nodes to degrade the small amount of absorbed test article. Long-term studies in rats given polypentosan sulfate and copovidone indicated that the histiocytosis does not progress to any pathological lesion; thus, the Committee concluded that the histiocytosis observed with calcium lignosulfonate (40–65) does not represent an adverse effect. The NOEL in the 90-day study was therefore the target dose of 2000 mg/kg bw per day.

The genotoxicity of calcium lignosulfonate (40–65) was evaluated in an assay for mutation in *Salmonella typhimurium* and *Escherichia coli,* with and without metabolic activation, and in a test for chromosomal aberration in Chinese hamster cells. No evidence of genotoxicity was found.

In a study of developmental toxicity, pregnant female Wistar rats were given diets providing calcium lignosulfonate (40–65) at a target dose of 0, 100, 300 or 1000 mg/kg bw per day. No effects on the dams or fetuses were reported, and it was concluded that the NOEL for reproductive effects was 1000 mg/kg bw per day.

The results of older studies with lignosulfonic acid salts of uncertain purity and relative molecular mass are of limited relevance to the safety assessment of calcium lignosulfonate (40–65).

Assessment of dietary exposure

The amount of calcium lignosulfonate (40–65) added for use as a carrier of carotenoids and fat-soluble vitamins is expected to be limited for technological reasons — for example, saturation of colouring effects — or by food regulations that limit the level of addition of vitamins to food. Use will also be limited by the ratio of the fat-soluble vitamins or carotenoids to carrier, proposed to be in the range from 1:5 to 1:200, the ratio used depending on the individual fat-soluble vitamin or carotenoid.

There were no poundage data available or data on current use levels of calcium lignosulfonate (40–65) in different food categories. Predictions of maximum dietary exposure were derived by the manufacturer by assuming that the amount of nutrient consumed was at the tolerable upper level of intake (UL) for the fat-soluble vitamins[1] or maximum predicted intakes for each carotenoid and applying the relevant ratio of use of the individual fat-soluble vitamin or carotenoid to the carrier.

Potential maximum levels of dietary exposure to calcium lignosulfonate (40–65) when used as a carrier for carotenoids for food uses ranged up to 95 mg/day or up to 2 mg/kg bw per day; and for use in supplements, from 5 to 125 mg/day or up to 2 mg/kg bw per day, assuming a body weight of 60 kg. It was considered unlikely that more than one carotenoid would be used in any one food; therefore, total maximum dietary exposures would likely be at the upper end of the range reported — i.e. 95 mg/day for food uses and 125 mg/day for use in supplements. It was reported that canthaxanthin was used as a colour in only one specific food and β-apo-8′-carotenal had limited uses compared with lycopene and β-carotene.

Estimates of potential dietary exposure to calcium lignosulfonate (40–65) from use as a carrier for fat-soluble vitamins in food ranged from 1 to 10 mg/day for vitamin D. There were no expected food uses for vitamin A, E or K. Estimates of dietary exposure to calcium lignosulfonate (40–65) from use as a carrier for fat-soluble vitamins in supplements ranged from 1 to 300 mg/day, or 0.02–5 mg/kg bw per day, assuming a body weight of 60 kg. The higher level of 500 mg/day for vitamin K was related to the UL for vitamin K established in Japan rather than actual intakes, which were not expected to exceed 10 mg/day. The highest potential dietary exposure for calcium lignosulfonate (40–65) as a carrier for individual nutrients in supplements was for supplements containing vitamin E at 300 mg/day, calculated by applying the relevant ratio of use for vitamin E to calcium

[1] The UL for food and supplements is the highest level of a nutrient that is likely to pose no adverse risk to almost all individuals for the population group. In this case, the highest UL for each nutrient set for any population was used to predict potential dietary exposures to calcium lignosulfonate (40–65).

lignosulfonate (40–65) to the UL for vitamin E. However, the manufacturers predict that maximum dietary exposure to calcium lignosulfonate (40–65) in multivitamin supplements could reach 400 mg/day or 7 mg/kg bw per day, assuming they contain all four vitamins, A, D, E and K, and assuming a body weight of 60 kg. It is likely that potential dietary exposures to calcium lignosulfonate (40–65) as a carrier for carotenoids or fat-soluble vitamins were overestimated, as use is limited to only the powdered form of the individual fat-soluble vitamin or carotenoid (50% of the total amount of carotenoids produced, 35–50% of the total amount of fat-soluble vitamins produced), not all these uses will be suitable for calcium lignosulfonate (40–65) as a carrier and there may be alternative carriers available.

Evaluation

In a metabolic study in rats, calcium lignosulfonate (40–65) was found to be poorly absorbed from the gastrointestinal tract. However, owing to the limitations of the study, it is difficult to determine the extent to which material of low molecular weight may be absorbed.

The toxicity data on calcium lignosulfonate (40–65) consist of a 28-day and a 90-day study of toxicity, negative results from a study of genotoxicity in vitro and a study of developmental toxicity that showed no adverse effects in either dams or fetuses. The NOEL for developmental toxicity in this study was 1000 mg/kg bw per day, the highest dose tested. In the 28-day study, inflammation of the rectum was observed, but this effect was not seen in the more extensive 90-day study. In the 90-day study, all the treated groups of animals displayed histiocytosis in the mesenteric lymph nodes, and the incidence of this effect increased with increasing dose. The histiocytosis seen in the mesenteric lymph nodes of rats treated with calcium lignosulfonate (40–65) has been observed with other substances of high molecular weight, such as polypentosan sulfate and copovidone (a copolymer of vinylpyrrolidone and vinyl acetate). Long-term studies with these substances in rats indicated that the histiocytosis does not progress and is not associated with carcinogenesis.

On the basis of the available data, the Committee concluded that the histiocytosis in the mesenteric lymph nodes of rats fed calcium lignosulfonate (40–65) is of no toxicological consequence; thus, the NOEL in the 90-day study is the target dose of 2000 mg/kg bw per day. The Committee therefore established an ADI of 0–20 mg/kg bw based on the NOEL of 2000 mg/kg bw per day from the 90-day study and application of a safety factor of 100. The 100-fold safety factor was considered by the Committee to be appropriate in the case of calcium lignosulfonate (40–65), despite the absence of a long-term study, because of its poor absorption, lack of toxicity in the 90-day study

and lack of evidence for developmental toxicity. In comparison with the ADI of 0–20 mg/kg bw, the maximum potential dietary exposure to calcium lignosulfonate (40–65) was low and not expected to exceed 7 mg/kg bw per day from use as a carrier of fat-soluble vitamins and carotenoids in food and supplements.

New specifications and a Chemical and Technical Assessment were prepared.

A toxicological monograph was prepared.

3.1.3 *Ethyl lauroyl arginate*

Explanation

This substance was placed on the agenda under the name "lauric arginate ethyl ester". The Committee decided that "ethyl lauroyl arginate" should be the name under which it would be evaluated. Ethyl lauroyl arginate was evaluated by the Committee at its present meeting at the request of CCFA at its Thirty-ninth Session (*4*). The Committee was asked to evaluate all data necessary for the assessment of the safety, dietary intake and specifications of ethyl lauroyl arginate. The Committee had not previously evaluated ethyl lauroyl arginate.

In 2007, the European Food Safety Authority (EFSA) established an ADI for ethyl lauroyl arginate of 0.5 mg/kg bw per day (*6*). On 1 September 2005, the United States FDA issued a letter indicating that it had no questions regarding a Notice that ethyl lauroyl arginate is GRAS (Notice No. GRN 000164) for use as an antimicrobial agent at concentrations of up to 225 mg/kg in the categories specified (*7*).

The Committee received a submission containing unpublished information on ethyl lauroyl arginate, including studies on N^{α}-lauroyl-L-arginine and a commercial formulation containing 19.5% ethyl-N^{α}-lauroyl-L-arginate hydrochloride (HCl) and 73% propylene glycol. Some of the results of these studies have been published in the open literature. A search of the scientific literature was conducted, but no additional information was identified.

Chemical and technical considerations

Ethyl lauroyl arginate is synthesized by first esterifying L-arginine with ethanol to obtain ethyl arginate HCl, which is then reacted with lauroyl chloride to form the active ingredient ethyl-N^{α}-lauroyl-L-arginate HCl. Ethyl-N^{α}-lauroyl-L-arginate HCl, which is present in the product in the range of 85–95%, is a cationic surfactant that has a wide spectrum of activity against bacteria, yeasts and moulds. N^{α}-lauroyl-L-arginine, a by-product in the manufacture of ethyl-N^{α}-lauroyl-L-arginate HCl, is also formed by enzymatic

action in fresh food. The intended use of ethyl lauroyl arginate is as a food preservative to prevent microbial growth and spoilage in a range of foods and drinks, to be used at concentrations of up to 225 mg/kg.

Toxicological data

The metabolism of ethyl lauroyl arginate has been well characterized. Studies with radiolabelled ethyl lauroyl arginate in vitro and in vivo show that it is well absorbed and rapidly metabolized by hydrolysis of the ethyl ester and lauroyl amide, via N^{α}-lauroyl-L-arginine and, to a lesser extent, L-arginine ethyl ester, to arginine, lauric acid and ethanol. Arginine subsequently undergoes normal amino acid catabolism via the urea and citric acid cycles, with ultimate elimination as carbon dioxide in the expired air and urea in the urine. Lauric acid enters normal fatty acid metabolism, and ethanol is converted to acetate, which enters normal biochemical pathways. Both lauric acid and ethanol are also present naturally in foods. After administration of [^{13}C]ethyl lauroyl arginate, the dose-corrected area under the plasma concentration–time curve for N^{α}-lauroyl-L-arginine in humans was 60-fold that in rats. The plasma concentrations of arginine were higher than those of N^{α}-lauroyl-L-arginine, indicating that most of the ethyl lauroyl arginate is metabolized before absorption. Given the rapid degradation of ethyl lauroyl arginate, exposure to this compound and N^{α}-lauroyl-L-arginine in vivo is likely to be short.

Ethyl lauroyl arginate is of low acute toxicity. In a 13-week feeding study in rats, the major observations were forestomach changes, such as erosions, ulcerations and epithelial hyperplasia, indicating an irritant action, at dietary concentrations of 15 000 mg/kg and greater. In addition, body weight gain and leukocyte counts were significantly decreased in males but not in females. No adverse effects were observed with ethyl lauroyl arginate at a dietary concentration of 5000 mg/kg, equal to 384 mg/kg bw per day. In another 13-week study in rats given diets containing a formulation of 19.5% ethyl-N^{α}-lauroyl-L-arginate HCl in propylene glycol, body weight gain and leukocyte counts were significantly decreased in females, but not in males, at dietary concentrations of 12 800 and 50 000 mg/kg, equal to 208 and 766 mg/kg bw per day. No treatment-related changes were observed by histopathological examination.

Decreased food consumption and body weight gain were observed in rats that were given ethyl lauroyl arginate at dietary concentrations of 6000 or 18 000 mg/kg for 52 weeks; these findings are likely to have been due to reduced palatability of the diet. Ethyl lauroyl arginate caused a dose-related irritation of the mucosal tissue of the forestomach, which was statistically significantly different from controls, at 18 000 mg/kg, but not at 6000 or 2000 mg/kg. A

reduction in the concentration of leukocytes in the peripheral blood was seen at all doses at 26 weeks and was dose related in females but not in males. At 52 weeks, the decrease in leukocytes was statistically significant compared with controls in males but not in females. These differences were due to lower concentrations of neutrophils or lymphocytes with occasional effects on monocytes and large unstained cells, with no consistent pattern of changes in leukocytes. In addition, evidence of neurobehavioural effects (higher low- and high-beam motor activity) was seen in the male rats at 18 000 mg/kg. In the absence of other evidence for an effect on the nervous system, this higher level of exploratory behaviour was considered of doubtful association with treatment and not indicative of neurotoxicity.

The Committee concluded that the changes seen in the stomach represented local irritation in the forestomach caused by storage of ingested diet and were thus not indicative of systemic toxicity. The Committee noted that the observed effects on leukocytes were inconsistent within and between studies and were not likely to be biologically significant. Furthermore, the changes were not accompanied by histopathological changes in the progenitor cell populations of the bone marrow or lymphoid tissue, which would be expected if the effect were due to systemic toxicity. Therefore, the Committee concluded that the highest dietary concentration tested, 18 000 mg/kg (equal to average doses of ethyl lauroyl arginate of approximately 900 mg/kg bw per day in male rats and 1100 mg/kg bw per day in female rats), was the NOAEL for systemic toxicity.

A range of studies in vitro (bacterial mutation, cytogenetics and gene mutation in mouse lymphoma cells) with ethyl lauroyl arginate and N^{α}-lauroyl-L-arginine did not provide evidence of genotoxicity.

In two studies of reproductive toxicity in rats, ethyl lauroyl arginate at a dietary concentration of 15 000 mg/kg delayed vaginal opening by 4 days in the female offspring. Although this effect was not accompanied by functional changes, the Committee considered this effect to be potentially adverse and concluded that the NOAEL for the dams was a dietary concentration of 6000 mg/kg, corresponding to 502 mg/kg bw per day expressed as ethyl lauroyl arginate, or 442 mg/kg bw per day expressed as the active component, ethyl-N^{α}-lauroyl-L-arginate HCl. Studies of potential developmental effects have been conducted in rats and rabbits given ethyl lauroyl arginate by oral gavage during pregnancy. The material used in these studies did not meet the proposed specifications for the content of the active ingredient. There were no adverse effects on fetal survival or development. Respiratory distress reported in some rats and rabbits at higher doses was considered to be an artefactual effect resulting from gavage dosing with the irritant solution and thus was not considered to be of relevance for dietary exposure.

Long-term studies of carcinogenicity were not available. However, the absence of pre-neoplastic lesions in the 52-week study and the absence of genotoxic activity do not suggest that ethyl lauroyl arginate has carcinogenic potential.

Assessment of dietary exposure

The Committee evaluated data submitted by the sponsor, as well as published information on an evaluation of ethyl lauroyl arginate completed by EFSA. Additionally, the Committee prepared international estimates of dietary exposure using GEMS/Food cluster diets.

Ethyl lauroyl arginate is used in many food types, with a maximum level for the active ingredient of 200 mg/kg. Carbonated beverages could be treated at concentrations of up to 100 mg/kg. The Committee noted that use levels based on the active ingredient are approximately 15% lower than those based on the article of commerce (i.e. the use level for the article of commerce is up to 225 mg/kg).

The current GEMS/Food international diets, derived from 13 clusters, were used to prepare international estimates of dietary exposure. They ranged from 1.0 (cluster J) to 4.5 (cluster B) mg/kg bw per day. A few food types not expected to contribute significantly to the overall dietary exposure were not included in the international estimates.

The sponsor submitted an estimate of dietary exposure to ethyl lauroyl arginate using data on food consumption from the USA. The mean dietary exposure to ethyl lauroyl arginate for the general population in the USA would be 3.0 mg/kg bw per day, and consumption at the 90th percentile would be 5.6 mg/kg bw per day.

The Committee noted that EFSA reviewed the safety of ethyl lauroyl arginate in a variety of food matrices in 2007. Using the Dose Adjustment For Normal Eating (DAFNE) database, the mean dietary exposure ranged from 0.14 mg/kg bw per day (France) to 0.50 mg/kg bw per day (Luxembourg), with an overall average of 0.32 mg/kg bw per day. Using individual dietary records from the United Kingdom, the mean dietary exposure ranged from 0.11 mg/kg bw per day in the elderly to 0.83 mg/kg bw per day in children aged 1.5–4.5 years. At the 97.5th percentile, dietary exposure ranged from 0.37 mg/kg bw per day in the elderly to 2.9 mg/kg bw per day in children aged 1.5–4.5 years.

The Committee noted for comparison that treatment of all solid food in the diet (default value, 1500 g/day from the USA) at 200 mg/kg would result in a dietary exposure of 5 mg/kg bw per day. Including treatment of carbonated beverages at 100 mg/kg (default value, 500 g/day from the USA) would make

the total theoretical maximum 6 mg/kg bw per day. These data are summarized in Table 3.

Table 3

Estimated dietary intake of ethyl lauroyl arginate (as ethyl-N$^{\alpha}$-lauroyl-L-arginate HCl)

Source	Mean dietary intake (mg/kg bw per day)	High-percentile dietary intake (mg/kg bw per day)
GEMS/Food	1–5	–
Sponsor	3.0	5.6[a]
EU – DAFNE[b]	0.32 (0.14–0.50)	–
EU – United Kingdom[b]	0.11–0.83	0.37–2.9[c]
Theoretical maximum	–	6

EU, European Union

[a] 90th percentile.

[b] Unclear if these data are expressed as ethyl lauroyl arginate or as ethyl-*N*$^{\alpha}$-lauroyl-L-arginate HCl.

[c] 97.5th percentile.

Evaluation

The majority of effects reported at high dietary concentrations of ethyl lauroyl arginate are considered to be related to its irritant action and not relevant to dietary exposure resulting from use as a food preservative. In two studies of reproductive toxicity in rats, administration of ethyl lauroyl arginate at a dietary concentration of 15 000 mg/kg resulted in delayed vaginal opening among the female offspring. Although this effect was not accompanied by functional changes, the Committee considered it to be adverse and concluded that the NOAEL for this effect was a dietary concentration of 6000 mg/kg, corresponding to 442 mg/kg bw per day expressed as ethyl-*N*$^{\alpha}$-lauroyl-L-arginate HCl, which should be used as the basis for establishing an ADI.

The Committee established an ADI of 0–4 mg/kg bw for ethyl lauroyl arginate, expressed as ethyl-*N*$^{\alpha}$-lauroyl-L-arginate HCl, based on the NOAEL of 442 mg/kg bw per day identified in studies of reproductive toxicity and a safety factor of 100.

The Committee noted that some estimates of high-percentile dietary exposure to ethyl lauroyl arginate exceeded the ADI, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

A new specifications monograph, Chemical and Technical Assessment and toxicological monograph were prepared.

3.1.4 *Paprika extract*

Explanation

At its fifty-fifth meeting in 2000 (Annex 1, reference *149*), the Committee concluded that paprika oleoresin is acceptable as a spice, confirming the outcome of an evaluation performed by the Committee at its fourteenth meeting in 1970 (Annex 1, reference *22*), which stated that the product was derived from a widely consumed natural foodstuff and there were no data indicative of a toxic hazard. The use as a spice was considered to be self-limiting and obviated the need for an ADI. Paprika extract was placed on the agenda of the present meeting at the request of the Thirty-ninth Session of CCFA for assessment of safety as a food colour, specification and exposure (*4*). CCFA asked if the existing safety assessment and specification for paprika oleoresin for use as a spice could be extended to the use as a food colour.

Since the source material and the manufacturing process differ for paprika preparations used as a spice and as a food colour, the name "paprika extract" was adopted for use as a food colour, leaving the term "paprika oleoresin" for use as a spice. The Committee was aware that the paprika preparations used for food colouring that are currently available in the marketplace may be referred to as paprika oleoresin. The Committee evaluated the use of paprika extract as a food colour.

Chemical and technical considerations

Paprika extract is obtained by solvent extraction of the dried ground fruit pods of *Capsicum annuum.* The major colouring principals are capsanthin and capsorubin. Other coloured compounds such as other carotenoids are also present. In addition to carotenoids and capsaicinoids, the extract contains mainly oil and neutral lipids, including tocopherols derived from fruit tissues and seeds of the dry material. Traces of volatiles may also be present; however, most of them are removed during processing when the solvents are removed. Some carotenoids are present as fatty acid esters. Paprika extracts have a very low content of capsaicin, in contrast to paprika products used as flavouring agents. Extracts are slightly viscous, homogeneous red liquids and are used to obtain a deep red colour in any food that has a liquid/fat phase. Typical use levels are in the range of 1–60 mg/kg finished food, calculated as colouring matter.

Toxicological data

There are no indications that carotenoids from paprika extract would behave differently from other oxygenated carotenoids with respect to their bioavailability.

Male and female rats were given paprika extract with a carotenoid content of 7.5% and a capsaicin content of less than 0.01% at dietary levels of up to 5%, equivalent to 3000 mg/kg bw, for 13 weeks without significant adverse effects. This finding was supported by other short-term studies in mice and rats given crude *Capsicum* extracts, where no adverse effects or only slight hyperaemia of the liver after 60 days of exposure was reported.

In a recently completed long-term combined 52-week study of toxicity and 104-week study of carcinogenicity, rats given diets containing up to 5% paprika extract (composition as described above) showed no evidence of toxicity or carcinogenicity at the highest dose tested.

A number of long-term studies of carcinogenicity in rodents have investigated various preparations of paprika and chilli and extracts of unspecified composition from two *Capsicum* species (*C. annuum* and *C. frutescens*). These long-term studies demonstrated no evidence that compounds extracted from *Capsicum* species are carcinogenic in experimental animals.

The historical literature on the mutagenicity and genotoxicity of extracts of chilli peppers and of various samples of capsaicin itself shows varied and often contradictory results. Nonetheless, the more recent studies using short-term tests considered in the present assessment clearly showed that pure capsaicin is not genotoxic.

While reports of epidemiological studies conducted in India and Mexico indicated an increased risk of gastric cancer in individuals who consumed large quantities of chilli peppers, these studies had limitations, including potential misclassification of subjects by exposure, large statistical imprecision of some of the analyses, lack of control of confounding factors and possible recall bias. Moreover, the relevance of these studies on consumption of chilli pepper to the use of paprika extract as a food colour is uncertain.

The Committee noted that there were no studies of reproductive toxicity with paprika extract.

Assessment of dietary exposure

Paprika extract is used in a wide range of foods as a colour. There were limited data on potential dietary exposures to total carotenoids from use of paprika extract as a food colour. Some data were available on dietary exposure to total carotenoids from consumption of fresh, dried peppers and chilli peppers. These data were used to put potential dietary exposure to total carotenoids from use of paprika extract as a food colour into the context of the whole diet.

Production data for Europe on the amount of paprika oleoresin sold for use as a food colour and as a spice were made available to the Committee at its

present meeting by the European Association for Manufacturers and Exporters of Pimentos and Derivatives (AFEXPO). Of the 1210 tonnes of paprika oleoresin sold annually, 16% was reported to be used as a food colour. Assuming that 7% of the paprika extract was total carotenoids and assuming a European population of 730 million, this resulted in a potential per capita mean dietary exposure to total carotenoids from use of paprika extract as a food colour of 0.05 mg of total carotenoids per day.

Estimates of dietary exposure to total carotenoids from use of paprika extract as a food colour were available for French and United Kingdom consumers. These were based on data on food consumption from the French Household Economic Survey, the United Kingdom National Diet and Nutrition Surveys and the 2007 Natural Food Colours Association (NATCOL) survey of use levels. Assuming that 7% of the paprika extract was total carotenoids, the estimated mean population dietary exposures to total carotenoids were 2–7 mg/day. For high consumers in France, estimated population dietary exposure to total carotenoids was 7 mg/day, assuming high consumption of foods containing paprika extract for two food categories at the 97.5th percentile of exposure and at a mean level for all other food groups. Estimated dietary exposures to total carotenoids for high consumers in the United Kingdom at the 95th percentile of exposure ranged from 6 to 13 mg/day.

The potential dietary exposure to total carotenoids from use of paprika extract as a colour from national survey data for France and the United Kingdom were in the same order of magnitude as the per capita mean dietary exposures to total carotenoids predicted from FAO food balance sheet data from consumption of fresh and dried peppers and chillies: i.e. France, 1–4 mg/day; and United Kingdom, 2–5 mg/day (assuming a concentration of 5000–13 000 mg total carotenoids/kg dry weight and a conversion factor of 20 for fresh peppers and 2 for dried peppers to dry weight). However, for countries with a much higher use of peppers and chillies in the diet, the per capita mean dietary exposure to total carotenoids predicted from FAO food balance sheet data from consumption of fresh and dried peppers and chillies was up to 60 mg/day (at concentrations of 5000 mg/kg dry weight) or 160 mg/day (at concentrations of 13 000 mg/kg dry weight).

Limited data were available on the potential dietary exposure to capsaicin from the use of paprika extract as a food colour. Dietary exposure to capsaicin could be predicted from estimates of dietary exposures to total carotenoids by applying a ratio of capsaicin content to total carotenoid content.

Evaluation

The concentration of capsaicin in paprika extracts is to be controlled by the specifications. Concern has been expressed in the past that capsaicin may be

carcinogenic; however, older long-term studies with capsaicin do not appear to provide evidence for carcinogenicity, and recent studies show that pure capsaicin is not genotoxic. The epidemiological studies reporting a relationship between consumption of chilli pepper and increased risk of gastric cancer have considerable limitations, which preclude the drawing of any definitive conclusion. Moreover, the Committee expressed the view that these studies were not relevant to the assessment of paprika extract used as a food colour.

In a well conducted 90-day study in rats given diets containing a commercial sample of paprika extract, no adverse effects were reported at a dietary concentration of 5%, equivalent to 3000 mg/kg bw. Similarly, in a long-term study of combined toxicity/carcinogenicity in rats given the same material, no evidence of toxicity or carcinogenicity was noted at dietary concentrations of up to 5%.

The Committee expressed concern as to whether the material tested in the 90-day and long-term studies was representative of all commercial production of paprika extract. The fact that the material tested contained less than 0.01% capsaicin and the fact that the Committee did not receive adequate data to establish a limit for capsaicin in the specifications for paprika extract added to this concern. The Committee requested data pertaining to the composition and capsaicin content of various commercial samples and information as to whether the material used in the toxicological tests was representative of all the products in commerce.

New specifications were prepared and made tentative pending the receipt of additional information on paprika extract, including concentrations of capsaicin and additional information about the composition of batches of extract produced by a variety of manufacturers. Therefore, the Committee did not allocate an ADI.

The Committee noted that there were existing specifications for paprika oleoresin with functional uses as both a colour and a flavouring agent. In response to the call for data for the present meeting, the Committee received data on the use of paprika preparations as a colour and as a result had no information to allow it to revise the existing specifications for paprika oleoresin. The Committee decided that the specifications for paprika oleoresin should be revised to emphasize its use as a flavour.

In addition to the new tentative specifications, a toxicological monograph and a Chemical and Technical Assessment were prepared.

3.1.5 *Phospholipase C expressed in* Pichia pastoris

Explanation

At the request of CCFA at its Thirty-ninth Session in 2007 (*4*), the Committee evaluated a preparation containing the enzyme phospholipase C (systematic name, phosphatidylcholine cholinephosphohydrolase; EC 3.1.4.3) from a genetically modified strain of *Pichia pastoris*. Phospholipase C has not been evaluated previously by the Committee. Phospholipase C catalyses the hydrolysis of phosphodiester bonds at the *sn*-3 position in glycerophospholipids (including phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine) to 1,2-diacylglycerol and the corresponding phosphate esters. Phospholipase C is to be used in refining vegetable oils intended for human consumption.

Genetic modification

Phospholipase C is produced by pure culture fermentation of a genetically modified strain of *P. pastoris*, which expresses the phospholipase C gene derived from DNA purified from a soil sample. The phospholipase C gene was sequenced and shown to be devoid of DNA sequences associated with haemolytic activity characteristic of certain microbial phospholipases.

Pichia pastoris is a methylotrophic yeast, which is not known to be associated with a disease of humans or animals. The phospholipase C production strain was constructed by transformation of the *P. pastoris* host strain SMD1168 with a purified DNA fragment containing multiple copies of the phospholipase C gene, the *P. pastoris HIS4* gene and non-coding DNA sequences necessary for expression of both genes; and insertion of the DNA fragment into a predetermined location in the *P. pastoris* genome. The *P. pastoris HIS4* gene encodes histidinol dehydrogenase and serves as a selectable marker to identify the transformed cells. The DNA fragment used in transformation was inserted at the alcohol oxidase 1 (*AOX1*) locus by homologous recombination.

Chemical and technical considerations

Phospholipase C is produced by pure culture fed-batch fermentation of the phospholipase C production strain. The fermentation medium consists of food-grade materials and contains glycerol as primary carbon source. After the cellular mass has reached a desired density, methanol is added to induce the expression of phospholipase C. The enzyme is secreted into the fermentation medium and is subsequently recovered by purification and concentration. The purified enzyme concentrate is formulated and standardized to a desired activity. Methanol is removed during purification steps, and its

residues in the final product are less than 9 mg/l. The phospholipase C enzyme preparation is a yellow or brown liquid, which typically contains 7% TOS.

The phospholipase C enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (Annex 1, reference *184*). It will be used in refining vegetable oils to hydrolyse phospholipids, primarily phosphatidylcholine and phosphatidylethanolamine, present in the crude oil. The resulting esters, phosphorylcholine and phosphorylethanolamine, as well as phospholipase C itself, will be removed from the oil during subsequent purification steps, whereas 1,2-diacylglycerol, which is also formed as a result of phospholipid hydrolysis, will remain in the oil.

Biochemical aspects

Phospholipase C from *P. pastoris* was tested for haemolytic activity using phospholipase C from *Clostridium perfringens* as a positive control. No haemolytic activity was detected.

Phospholipase C was also evaluated for potential allergenicity according to the bioinformatics criteria recommended by FAO/WHO (*5*). The amino acid sequence of phospholipase C was compared with the amino acid sequences of known allergens. No sequence homology that would suggest that phospholipase C is an allergen was identified.

Toxicological data

Toxicological studies were performed with the phospholipase C enzyme using a representative batch (PLC-16449-PD267B), which was produced according to the procedure used for commercial production. The liquid enzyme concentrate was lyophilized to produce the final, non-formulated test substance with an average activity of 315 U/mg (where a unit is defined as the quantity of the enzyme that hydrolyses 1 μmol of phosphatidylcholine per minute at 37 ºC and pH 7.3) and a TOS value of 83.6% (w/w). Before being used in toxicological studies, phospholipase C was analysed to demonstrate that it conformed to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (Annex 1, reference *184*).

In a 13-week study of general toxicity in rats, no significant treatment-related effects were seen when the phospholipase C enzyme was orally administered at doses of up to 2000 mg/kg bw per day by gavage. Therefore, the NOEL was identified as 1672 mg TOS/kg bw per day, the highest dose tested. Phospholipase C enzyme was not mutagenic in an assay for mutagenicity in bacteria in vitro and was not clastogenic in an assay for chromosomal

aberration in mammalian cells in vitro. Similarly, an assay for micronucleus formation in mice showed no evidence of a clastogenic effect in vivo.

Assessment of dietary exposure

An estimate of dietary exposure to phospholipase C was made by the Committee based on the 13 cluster diets of the GEMS/Food categorization and on the Concise European Food Consumption Database for the adult population (aged 16–64 years). The European database compiles mean and high percentiles of individual food consumption for 15 broad food categories from the majority of European countries (n = 17). The GEMS/Food database contains per capita daily consumption of food commodities. In these estimates, reported consumption data have been combined with the maximum use level recommended by the sponsor, 1000 mg of the commercial enzyme preparation (7% TOS content) per kilogram of vegetable oil. For the GEMS/Food data, the food categories used in the calculation were vegetable oils and fats, including olive, coconut, cotton seed, groundnut, linseed, maize, palm kernel, rape seed, sesame seed, soya bean, sunflower and other oils of vegetable origin, butter of karité and margarine. For the European database, the food category used was the "fat products" category, including mayonnaise, dressings, béchamel and hollandaise sauces, low-fat dressings or mayonnaise, goose fat and coconut extract.

Mean consumption of vegetable oils ranged on average from 9 to 68 g/day (GEMS/Food cluster diets; includes the range 21–59 g/day in Europe). For high-percentile (95th percentile) consumers in Europe, consumption of vegetable oils ranged from 51 to 150 g/day. If the enzyme is not removed from the oil and is used at proposed levels, the potential mean dietary exposure to phospholipase C from *P. pastoris*, assuming a body weight of 60 kg, would be 0.011–0.079 mg TOS/kg bw per day, and the potential dietary exposure for high consumers would be 0.059–0.175 mg TOS/kg bw per day.

Evaluation

Comparing the conservative exposure estimates with the NOEL of 1672 mg TOS/kg bw per day from the 13-week study of oral toxicity, the margin of exposure is generally more than 10 000. The Committee allocated an ADI "not specified" for phospholipase C expressed in *P. pastoris*, used in the applications specified and in accordance with good manufacturing practice.

A toxicological monograph was prepared.

A Chemical and Technical Assessment and new specifications were prepared.

3.1.6 *Phytosterols, phytostanols and their esters*

Explanation

Phytosterols, phytostanols and their esters were evaluated by the Committee at its present meeting at the request of CCFA at its Thirty-ninth Session (*4*). Phytosterols and phytostanols are substances that are similar in structure to cholesterol and are formed exclusively in plants. They are added to food for their blood cholesterol-lowering properties.

Phytosterols, phytostanols and their esters have not been evaluated previously by the Committee. In 2000 (*8*) and again in 2002 (*9*), the former Scientific Committee on Food (SCF) of the European Commission assessed the safety of phytosterols in food. The United States FDA responded to several GRAS notices concerning specified uses of phytosterols and phytostanols in various types of food (http://vm.cfsan.fda.gov/~rdb/opa-gras.html#grastop).

This summary describes the data on phytosterols, phytostanols and their esters discussed at the present meeting, with the focus on newly submitted data and other new information published since the evaluations by other regulatory bodies.

The Committee noted that phytosterols, phytostanols and their esters do not fall into the definition of a food additive as defined by the Codex Alimentarius Commission (*10*),[1] because they do not fulfil a technological purpose in food or food processing. At its present meeting, the Committee evaluated the safety of these mixtures, when present in food. It is stressed that the effectiveness of these substances in reducing blood concentrations of cholesterol was not assessed by the Committee.

Chemical and technical considerations

Phytosterols, phytostanols and their esters are structurally related to cholesterol, but differ in the structure of the side-chain. Phytosterols have an unsaturated bond between positions 5 and 6 on the B-ring of the steroidal skeleton, while this bond is saturated in phytostanols. The more common phytosterols, β-sitosterol and campesterol, are found to varying degrees in soya bean oil and tall oil arising from wood pulping. Minor components, among them stigmasterol and brassicasterol, are also present in other vegetable oils. The major

[1] "*Food additive* means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include 'contaminants' or substances added to food for maintaining or improving nutritional qualities."

phytostanols are β-sitostanol and campestanol. Phytosterols and phytostanols are extracted from plant materials as the free form and as their fatty acid esters. There are numerous commercial products, both raw materials and finished products, containing phytosterols, phytostanols and their esters in different proportions.

Toxicological data

The bioavailability of phytosterols and phytostanols is lower than that of cholesterol. Absorption from the gastrointestinal tract in humans has been estimated to be about 5% for β-sitosterol, 15% for campesterol and less than 1% for β-sitostanol, campestanol and other phytostanols. In a recent human study, where deuterium-labelled substances were emulsified with lecithin and administered with the diet, even lower absorption rates (campesterol, 2%; β-sitosterol, campestanol and β-sitostanol, <1%) were observed. Studies in both rats and humans indicate that the bioavailability of phytosterols and phytostanols is influenced by the form of administration. Different methods used in absorption studies may partly explain the quantitative differences observed.

Three sets of toxicity data were submitted to the Committee:

1. studies with phytosterol ester mixtures derived from vegetable oil distillates (mainly soya bean). The main constituents were β-sitosterol (45–51%), stigmasterol (17–23%) and campesterol (26–29%), esterified with fatty acids from sunflower oil;

2. studies with a mixture of unesterified phytosterols and phytostanols, derived from tall oil, which consisted of β-sitosterol (about 40–65%), β-sitostanol (16–31%), campesterol (6–15%) and campestanol (2–11%);

3. studies with two types of phytostanol ester mixtures: wood-derived mixtures of phytostanol esters (with a stanol composition of about 94% β-sitostanol and about 6% campestanol) and vegetable oil–derived mixtures of phytostanol esters (with a stanol composition of about 68% β-sitostanol and about 32% campestanol).

Short-term studies of toxicity with phytosterol ester mixtures. In a 90-day study of toxicity, rats were fed diets containing phytosterol esters at a concentration of 0, 0.16, 1.6, 3.2 or 8.1% (w/w). These dietary concentrations were equal to phytosterol at 0, 0.08, 0.78, 1.6 and 3.9 g/kg bw per day for males and 0, 0.09, 0.87, 1.8 and 4.2 g/kg bw per day for females (mean intakes over the study period). Treatment-related effects observed were restricted to slight changes in haematological parameters (slight reduction in numbers of platelets, eosinophils, neutrophils and lymphocytes) and clinical chemistry values (increases in serum activity of alkaline phosphatase and alanine

aminotransferase). There were neither macroscopic findings at necropsy nor histological findings attributable to treatment with phytosterol esters. On the basis of the minimal changes noted and the absence of any histopathological changes, the NOEL was 8.1% phytosterol esters in the diet, equal to phytosterols at a dose of 3.9 g/kg bw per day, the highest dose tested.

In a 90-day study of toxicity, rats were given phytosterols isolated from soya beans and esterified with fatty acids from olive oil at a dose of 0, 1, 3 or 9 g/kg bw per day by gavage. Reduced body weight gain was observed in both sexes, and an increased incidence of cardiomyopathy was observed in males but not in females at the highest dose. Slight, reversible changes in haematological parameters occurred at the two highest doses and were not considered to be adverse effects. The lowest-observed-adverse-effect level (LOAEL) was 9 g/kg bw per day on the basis of effects observed at the highest dose. The NOAEL for phytosterols was 3 g/kg bw per day.

Short-term studies of toxicity with mixtures of phytosterols and phytostanols. Ninety-day studies of toxicity were available for two mixtures of phytosterols and phytostanols, which differed slightly in composition owing to different production processes (solvent extraction, vacuum distillation). In the first study, rats were fed a phytosterol/phytostanol mixture obtained by solvent extraction at a dietary concentration of 0, 1.25, 2.5 or 5%, equal to mean intakes of 0, 1.0, 2.0 and 4.2 g/kg bw per day for males and 0, 1.2, 2.4 and 4.8 g/kg bw per day for females over the study period. No clearly treatment-related effects were seen in this study at any dose, and the NOEL was 4.2 g/kg bw per day for this mixture of phytosterols and phytostanols.

The second study was carried out with a phytosterol/phytostanol mixture obtained by vacuum distillation. Rats were fed the mixture at a dietary concentration of 0, 1.25, 2.5 or 5%, equal to mean intakes of test material of 0, 0.99, 2.0 and 4.1 g/kg bw per day for males and 0, 1.1, 2.2 and 4.6 g/kg bw per day for females over the study period. No consistent treatment-related effects were observed, apart from some changes in clinical chemistry parameters in females, but not in males (increased activity of serum alanine aminotransferase and γ-glutamyl transferase and increased concentrations of urea). Although these observations may indicate early effects in the liver, no histopathological changes were observed in the liver. The NOEL was 4.1 g/kg bw per day.

Short-term studies of toxicity with phytostanol ester mixtures. In a 90-day study of toxicity, rats were given one of two mixtures of phytostanol esters of similar composition, which were derived from wood and from vegetable oil, respectively. Rats received feed containing the wood-derived mixture at a concentration of 0, 0.34, 1.7 or 8.4% (w/w) or the vegetable oil– derived mixture at a concentration of 0, 0.36, 1.8 or 8.9% (w/w), which correspond

to the same levels of phytostanols in the feed. Mean intakes of phytostanols from both mixtures were 0, 0.1, 0.5 and 2.7 g/kg bw per day for males and 0, 0.1, 0.6 and 3.0 g/kg bw per day for females over the study period. The most prominent treatment-related findings for both mixtures were decreases in plasma concentrations of vitamins E, D and K_1 in both sexes at the highest dose (about 8.5% phytostanol esters in the diet). Plasma concentrations of vitamin A and β-carotene were unaffected at all dietary concentrations. The influence of phytosterols and phytostanols on carotenoid and vitamin concentrations was also investigated in numerous studies in humans (see below). In several of these studies (with doses of up to 3 g/person per day), decreases in plasma concentrations of carotenoids (α-carotene, β-carotene, lycopene) and of α-tocopherol could be observed, but concentrations of vitamins A, D and K were unaffected. This is in contrast to the effects on vitamin concentrations observed in the study in rats mentioned above, which renders the significance of these findings for humans unclear. The effects on plasma concentrations of vitamins were not investigated in the 90-day study of toxicity with phytosterol esters; thus, it remained unclear whether the effects observed with phytostanol esters are unique to this mixture. Taking into consideration the fact that the respective effects on vitamin concentrations were not observed in studies in humans, the Committee concluded that these effects were not to be considered in this evaluation.

Studies of reproductive toxicity with phytosterol ester mixtures. In a two-generation study of reproductive toxicity, rats were fed diets containing phytosterol esters at concentrations of 0, 1.6, 3.2 and 8.1% (w/w), equal to 0, 0.5–2.3, 0.9–4.5 and 2.3–12.6 g/kg bw per day, respectively (ranges of weekly averages). The only treatment-related observations were slight, but significant, decreases in food consumption, food efficiency and body weight gain of F_0 and F_1 males and females at the highest dose. The viability index of pups at postnatal day 4 for F_0 and F_1 pups was slightly decreased, but no differences in pup mortality were observed when analysed on a litter basis, and pup weights of both generations were unaffected. The NOEL was 8.1% phytosterol esters in the diet, equal to 2.7 g/kg bw per day expressed as phytosterols (average exposure during premating and gestation for F_0 and F_1 females).

Studies of reproductive toxicity with phytostanol ester mixtures. In a two-generation study of reproductive toxicity, rats were given feed containing a mixture of phytostanol esters at a concentration of 0, 1, 2.5 or 5% phytostanols. Intakes of phytostanols in F_0 and F_1 were 0.6– 1.3, 0.4–0.7 and 1.0–2.1 g/kg bw per day for the low dose group females during premating, gestation and lactation, respectively (ranges of weekly averages). For the middle-dose females, intake levels were 1.5–3.4, 1.0–1.7 and 2.5–5.6 g/kg bw per day during premating, gestation and lactation,

respectively. For high-dose females, intake levels were 3.2–7.3, 2.1– 3.6 and 5.2–11.1 g/kg bw per day during premating, gestation and lactation, respectively. Intakes by F_0 and F_1 males during premating were 0.5–1.4, 1.3–3.5 and 2.8–7.7 g/kg bw per day for low-, middle- and high-dose animals, respectively. The only treatment-related effect observed were decreased pup body weights in both generations at the highest dose at postnatal days 14 and 21. Based on these effects observed in the highest dose group (dietary concentration 5%, equal to a LOAEL of 8.1 g phytostanols/kg bw per day, calculated as the average dose during lactation), the dietary concentration of 2.5% phytostanols was considered the NOAEL for reproductive and developmental toxicity in this study, which equals 4.1 g phytostanols/kg bw per day (average dose during lactation).

In a prenatal developmental toxicity study, a mixture of phytostanol esters was fed to female rats during gestation days 0–21 at concentrations of 0, 1.8, 4.4 and 8.8% phytostanol esters, which correspond to concentrations in the diet of 0, 1, 2.5 and 5% phytostanols. In the high-dose group, maternal body weights were transiently reduced. No treatment-related effects with respect to malformations or developmental toxicity were observed. The NOEL for developmental toxicity in this study was 3.2 g phytostanols/kg bw per day.

Genotoxicity. All mixtures were inactive in assays for gene mutations in vitro in bacteria and in mouse lymphoma cells and did not induce chromosomal aberrations in vitro in mammalian cells. Phytosterol esters and a mixture of phytosterol esters and phytostanol esters were inactive in assays for micronuclei induction in the bone marrow of rodents in vivo. Phytosterol esters did not induce unscheduled DNA synthesis in rat liver.

Estrogenicity. Phytosterols, a mixture of phytosterols and phytostanols, and phytostanols were investigated for possible estrogenic activity. They did not reveal uterotrophic activity in vivo in immature female rats. Phytosterols failed to show estrogenic activity in vitro in the competitive estrogen receptor binding assay and the recombinant yeast assay. Also, phytostanols did not induce proliferation of human mammary adenocarcinoma cells.

Human studies

In several double-blinded, placebo-controlled human studies, where subjects received diets containing added phytosterol esters or phytostanol esters, reduced plasma concentrations of carotenoids and α-tocopherol were noted. In these studies, phytosterol and phytostanol esters were administered over periods of 3–8 weeks. In most of these studies, no effects on (pro)vitamins were observable when concentrations were lipid adjusted. In a study of 1-year duration, serum concentrations of α-carotene, β-carotene, α-tocopherol and

lycopene were lower after phytosterol ester consumption (1.7 g phytosterols/day as esters in fat spread) in 185 healthy subjects, compared with controls. Decreases of lipid-adjusted α-carotene and β-carotene levels were statistically significantly higher in the exposed group. Another 1-year study investigated the effects on carotenoid levels after administration of sitostanol esters in fat spread (3.0 g β-sitostanol/day). Absolute plasma levels of α-carotene, β-carotene and α-tocopherol were significantly reduced. If normalized to cholesterol concentration, only reduction of the β-carotene plasma concentration was statistically significant. In some of the human studies, a possible influence on vitamins A, D and K was also investigated. Plasma concentrations of these vitamins were generally not affected by consumption of food enriched with phytosterols, phytostanols or their esters. This indicates that the marked effects on these vitamins observed in a 90-day study, where rats were fed diets with phytostanol esters, were not observed in humans, at least at dose levels applied in the human studies (1–3 g/day).

Available data show that diets containing added phytosterols, phytostanols or their esters in doses up to 2 g/day (as phytosterols/phytostanols) lead to (up to) 2-fold increases in plasma concentrations. Various epidemiological studies investigated a possible correlation between phytosterol plasma levels and indicators for atherosclerosis and an increased risk for coronary heart diseases. Taken together, to date there is no convincing evidence for an association of elevated phytosterol levels and increased risk for coronary heart diseases.

Assessment of dietary exposure

The Committee received and reviewed information on dietary exposure submitted by two sponsors, as well as published information from EFSA and the United States FDA. The relative molecular masses of the five major phytosterols and phytostanols — namely, campesterol and campestanol, β-sitosterol and β-sitostanol, and stigmasterol — range from 400 to 416 and have not been differentiated owing to the small relative differences among them. Also, the ratio between the relative molecular masses of the collective free phytosterols and phytostanols and their esters was taken to be 60% as a default (3.4 g of esterified phytosterols or phytostanols to metabolically deliver 2 g of free phytosterols or phytostanols).

Phytosterols, phytostanols and their esters are regulated or allowed for use in numerous countries, particularly in the European Union (EU), the USA, Australia and New Zealand, either as food additives/ingredients or as supplements. The cholesterol-lowering effects of free phytosterols and phytostanols are stated to reach a plateau at approximately 2 g/person per day. Consequently, food manufacturers have been formulating products containing free phytosterols and/or phytostanols so as to deliver a convenient “dose”,

requiring one, two or three standard portions a day to reach the 2-g level of intake (or, for the esterified products, 3.4 g). Rather than a single upper use level, such as "up to 50 mg/kg food", the products are individually prepared based on the typical or standard portions sold in a given jurisdiction. Many product types have been developed, including, but not limited to, margarines, yogurts and yogurt drinks, cheese products, dairy beverages, snack (power) bars, candy chews and orange juice. Other potential uses include baked goods and baking mixes; egg products; fats and oils; frozen dairy desserts and mixes; gelatins; ground coffee; grain products and pastas; gravies and sauces; hard candy; milk; milk products; puddings and pie fillings; soft candy; soups and soup mixes; and snack foods.

The natural background intake of free phytosterols and phytostanols from numerous plant products, including seeds, nuts and vegetable oils, has been estimated to be in the range of 150–400 mg/day, with the phytosterols representing approximately 90% of the total. As discussed above, consumers of foods containing phytosterols, phytostanols or their esters are directed to consume them in one, two or three portions a day in order to achieve a dose of 2 g free phytosterols or phytostanols per day (30 mg/kg bw per day for a 60-kg individual). The Committee was aware that products containing phytosterols, phytostanols or their esters are markedly more expensive than the same products without them (up to 5 times the cost for some products in the United Kingdom market) and concluded that inadvertent purchase and consumption of such products over a lifetime were highly unlikely. Therefore, the Committee concluded that dietary exposure to free phytosterols and phytostanols would typically be less than 30 mg/kg bw per day.

Evaluation

The Committee evaluated the toxicological studies with a range of phytosterols, phytostanols and their esters, together with several double-blinded, placebo-controlled human studies, in which these substances were added to the diet. As phytosterol and phytostanol esters and mixtures of phytosterols and phytostanols generally show similar effect profiles, the Committee considered establishing a group ADI.

Using the combined evidence from several short-term (90-day) studies of toxicity, the Committee identified an overall NOAEL[1] of 4200 mg/kg bw per day. The Committee considered the margin between this overall NOAEL and

[1] The Committee was aware of the definition of the overall NOAEL by the Joint FAO/WHO Meeting on Pesticide Residues (*11*): "When they [the studies] are comparable, including consideration of study design, endpoints addressed, and strain of animals, the overall NOAEL should be the highest value identified in the available studies that provides a reasonable margin (≥2) over the lowest LOAEL, provided that due consideration is given to the shape of the dose–response curve."

the lowest LOAEL from the 90-day toxicity studies of 9000 mg/kg bw per day as adequate for this overall NOAEL to be used as the basis for establishing an ADI. This conclusion is supported by the results of the available studies of reproductive toxicity.

The Committee established a group ADI of 0–40 mg/kg bw for the group of phytosterols, phytostanols and their esters, expressed as the sum of phytosterols and phytostanols in their free form, based on the overall NOAEL, to which a safety factor of 100 was applied. This safety factor incorporates a factor of 10 for interspecies differences and a factor of 10 for intraspecies differences. Based on the availability of a range of studies in humans, which includes two 1-year studies, the Committee considered the safety factor of 100 as sufficient to also account for deficiencies in the database, such as the absence of chronic studies in experimental animals. As there is no evidence for genotoxicity of phytosterols or phytostanols and their esters and no indication of potential for carcinogenicity from the available toxicity studies, the Committee did not see a need for a carcinogenicity study to be performed.

Based on available data, the Committee concluded that dietary exposure to phytosterols and phytostanols would typically be within the ADI range of 0–40 mg/kg bw.

A Chemical and Technical Assessment and new specifications were prepared. A toxicological monograph was prepared.

3.1.7 *Polydimethylsiloxane*

Explanation

Polydimethylsiloxane (PDMS) (synonyms: dimethylpolysiloxane, dimethicone) is widely used in foods as an antifoaming and anticaking agent.

PDMS was placed on the agenda by the JECFA Secretariat for consideration of the applicability of the current ADI of 0–1.5 mg/kg bw to the material currently in commerce. This ADI was established at the eighteenth meeting (Annex 1, reference *35*). When the Committee reviewed the ADI at its twenty-third meeting (Annex 1, reference *50*), it stated that this ADI applied to PDMS with 200–300 repeat subunits of $[(CH_3)_2SiO]$ (weight-average molecular weight range, 15 000–22 000) because of concern that the material of lower molecular weight might be more readily absorbed.

At its thirty-seventh meeting in 1990 (Annex 1, reference *94*), the Committee revised the specifications for material with a weight-average molecular weight range of 6800–30 000 (90–410 subunits) and a viscosity range of 100–1500 cSt (mm^2/s). However, the toxicological properties of this material were not re-evaluated. As a consequence, material with an average weight at

the lower end of this range was outside the limits covered by the previously established ADI.

The Committee at its present meeting considered new studies on the absorption of two PDMS products: a material with a viscosity of 10 cSt and a number-average molecular weight of 1000 and a material with a viscosity of 350 cSt and a number-average molecular weight of 10 000. New toxicological studies were also reviewed: short-term studies in rats fed diets containing one of these two materials at concentrations of 10 000–100 000 mg/kg diet and a long-term study of toxicity and carcinogenicity with the 10 cSt material administered at doses of 100–1000 mg/kg bw.

Toxicological data

Orally administered [^{14}C]PDMS with viscosities of 350 cSt and 10 cSt were excreted unchanged in the faeces, with little, if any, absorption.

Ocular lesions were consistently observed in the available short-term and long-term studies of toxicity with PDMS given by oral administration in the diet or by gavage. Dose-related ocular lesions (corneal opacities/crystals, granulomatous inflammation and suppurative inflammation of the corneal epithelium) were observed in 28-day and 13-week studies in F344 rats given 10 cSt and 350 cSt PDMS.

In the long-term study with 10 cSt PDMS in F344 rats, corneal opacity was observed at slightly increased incidences in males at 1000 mg/kg bw per day and in females at 100 or 1000 mg/kg bw per day. Corneal opacity usually correlated with the microscopic finding of keratitis or the incidental microscopic finding of corneal dystrophy. Inflammation of the nasolachrymal duct was observed with slightly increased incidence and severity in males at 1000 mg/kg bw per day. Food consumption, body weight gain, haematology and serum parameters were unchanged in all the groups treated with PDMS compared with the control group, and no other adverse effects were observed in short-term or long-term studies.

Assessment of dietary exposure

PDMS is used as an antifoaming agent in fruit and vegetable juices, an anticaking agent in confectionery and flour products, and an emulsifier in edible oils essentially free of water. It is included in the current version of the GSFA for use in a wide range of foods at acceptable maximum levels of 10–110 mg/kg food.

Budget method calculations indicate that the use of PDMS in solid food and beverages at the GSFA acceptable maximum levels may result in the temporary ADI of 0–0.8 mg/kg bw established at the present meeting being exceeded, assuming use in 25% of solid food and 25% of beverages. Hence, data on dietary exposures were required based on the current version of GSFA acceptable maximum levels for PDMS.

Poundage data were not available for PDMS. Potential mean dietary exposure for the Australian population based on individual dietary records was 11 mg/day (0.2 mg/kg bw per day) for consumers of foods containing PDMS; for high consumers (at the 90th percentile) of PDMS, dietary exposure was 27 mg/day (0.5 mg/kg bw per day). Potential mean dietary exposure for the New Zealand adult population was 10 mg/day (0.1 mg/kg bw per day); for high consumers (at the 90th percentile) of PDMS, potential dietary exposure was 26 mg/day (0.3 mg/kg bw per day). Major contributors to total potential dietary exposure were water-based flavoured drinks, alcoholic beverages, flour products, desserts, and fruit and vegetable preparations.

To determine whether these results were typical of other countries with similar levels of production of processed foods, potential dietary exposures to PDMS were estimated for 17 European countries using information on diets from the Concise European Food Consumption Database for the adult population aged 16–64 years, assuming PDMS was used at the GSFA acceptable maximum levels. Potential mean dietary exposures to PDMS for European populations ranged from 17 to 30 mg/day (0.2–0.4 mg/kg bw per day); for high consumers (95th percentile), potential dietary exposures ranged from 35 to 83 mg/day (0.5– 1.1 mg/kg bw per day). Major contributors were cereal and cereal products and non-alcoholic and alcoholic beverages. However, it should be noted that basing potential dietary exposures on the amounts of food consumed for 15 broad food categories will overestimate the dietary exposure to PDMS, as use is often restricted to specific subgroup categories within the broader food groups.

For Australia and New Zealand, potential mean dietary exposures to PDMS for consumers only of foods containing PDMS were 19% and 17% of the temporary ADI of 0–0.8 mg/kg bw, respectively; for 90th-percentile consumers, potential dietary exposures were 66% and 43% of the temporary ADI, respectively. Potential mean dietary exposures to PDMS for European countries were similar to those for Australia and New Zealand, although somewhat higher for high consumers (potential mean dietary exposure, 28–49% of the temporary ADI; high consumers' dietary exposure, 66–138% of the temporary ADI).

The limited data available indicate that there may be potential to exceed the temporary ADI for high consumers of PDMS; however, all dietary exposures are likely to be overestimates, as maximum levels of use for PDMS were assumed and, for the European countries, it was assumed that PDMS was used in broader food categories than those listed in the GSFA. In reality, this is unlikely, as alternative food additives will be used in some foods, and use levels may be lower than the acceptable maximum level.

Evaluation

Absorption studies on the 10 cSt and 350 cSt material indicated that neither product was absorbed to any significant extent. Also, the new toxicological studies did not reveal any significant differences between the two materials. However, the reports of the toxicological studies on which the ADI was established at the eighteenth meeting did not refer to any ocular effects, and it is unclear whether ophthalmological examinations were conducted. Conversely, in all the more recent short- and long-term studies of toxicity with PDMS reviewed at the present meeting, dose-dependent increases in the incidence and severity of ocular lesions were consistently observed after oral dosing both in the diet and by gavage. It was stated in the study reports that this seems to be a local irritant effect; however, it is unclear whether the eye might have been exposed topically to PDMS at a level causing irritation, particularly after administration by gavage. Furthermore, the Committee was aware that studies of ocular irritation conducted in relation to cosmetic use resulted in PDMS being classified as a mild to minimal irritant, but the material tested may have differed from that used in the studies in which PDMS was administered orally. The mechanism by which the ocular lesions arose is therefore unclear, although the lack of absorption of PDMS indicates that it is unlikely to be a direct systemic effect. Consequently, the relevance of the ocular lesions for food use of PDMS could not be determined.

The previously established ADI of 0–1.5 mg/kg bw was withdrawn. Using an additional safety factor of 2, the Committee established a temporary ADI of 0–0.8 mg/kg bw for PDMS, pending the results of studies to elucidate the mechanism and relevance of the ocular toxicity and provision of data on actual use levels in foods. The temporary ADI applies to PDMS that meets the revised specifications prepared at the present meeting. The temporary ADI will be withdrawn if the required data are not provided before the end of 2010.

A toxicological monograph was prepared

The existing specifications were revised. The section on definition was clarified, the Chemical Abstracts Service (CAS) number was corrected and the test for solubility and the sample preparation for an infrared absorption

spectrum were modified to eliminate the use of certain solvents. Other minor editorial changes were made.

3.1.8 *Steviol glycosides*

Explanation

Steviol glycosides are natural constituents of the plant *Stevia rebaudiana* Bertoni, belonging to the Compositae family. Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening properties.

At its fifty-first meeting, the Committee evaluated toxicological data on stevioside and the aglycone steviol (Annex 1, reference *137*) and specified further information needed. Based on new data and information, at its sixty-third meeting (Annex 1, reference *173*), the Committee determined that the commercial material should be known as "steviol glycosides" and established tentative specifications for material containing not less than 95% of the total of four specified glycosylated derivatives of steviol (i.e. stevioside, rebaudioside A, rebaudioside C and dulcoside A). Additionally, the sum of stevioside and rebaudioside A content was specified at not less than 70% of the four steviol glycosides.

Also at its sixty-third meeting, the Committee reviewed additional biochemical and toxicological data on the major steviol glycosides and on the aglycone steviol. The Committee noted that steviol glycosides are poorly absorbed and are metabolized by the intestinal microflora by successive hydrolytic removal of glucose units to the aglycone, steviol, which is well absorbed. Therefore, the toxicity of the glycosides was related to the steviol content. A temporary ADI of 0–2 mg/kg bw for steviol glycosides expressed as steviol was established on the basis of the NOEL[1] of 2.5% stevioside in the diet, equal to 970 mg/kg bw per day, or 383 mg/kg bw per day expressed as steviol, in a 2-year study in rats and with a safety factor of 200. In the groups at 5%, final survival rates in the males and body weight gain and absolute kidney weights in both sexes showed significant reductions compared with those in controls. The overall safety factor of 200 incorporated a factor of 2 related to the need for further information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. The Committee specified the need for studies involving repeated exposure of normotensive and hypotensive individuals and patients with type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes to dietary and therapeutic doses. This was because the evidence available at the time was inadequate to assess whether

[1] Before the sixty-eighth meeting, the Committee used the term NOEL to include the current definitions of both NOEL and NOAEL. According to the decision taken by the Committee at its sixty-eighth meeting (Annex 1, reference *187*), this NOEL would now be termed a NOAEL.

the pharmacological effects of steviol glycosides would also occur at dietary exposure levels, which could lead to adverse effects in some individuals (e.g. those with hypotension or diabetes).

Also at its sixty-third meeting, the Committee estimated international intakes of steviol glycosides to be in the range of 1.3 (African diet) to 3.5 mg/kg bw per day (European diet), expressed as steviol, assuming that all dietary sugars (total sugars and honey) are replaced by steviol glycosides. The Committee acknowledged that this was a conservative estimate and that actual intakes were likely to be 20–30% of this figure.

At its sixty-eighth meeting (Annex 1, reference *184*), the Committee considered the information that had become available since the sixty-third meeting. This comprised two submissions, which included a summary of published toxicological studies and some unpublished data, additional information identified from the scientific literature and responses intended to resolve the outstanding issues relevant to the specifications. The Committee was also informed that results of an ongoing toxicity testing programme, including clinical studies, would be available by August 2007. The Committee considered that the newly available data did not raise additional concerns regarding the safety of steviol glycosides, but that the ongoing clinical studies, which more closely addressed the requirements specified at the sixty-third meeting, would be essential for the evaluation. The Committee therefore extended the temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, pending submission of the results of the ongoing studies by the end of 2008.

Also at the sixty-eighth meeting, the existing tentative specifications were revised by requiring an assay of not less than 95% of the total of seven named steviol glycosides, by deleting the assay requirement for the sum of stevioside and rebaudioside A content to be not less than 70%, by adding pH as an identification test, by increasing the limit for loss on drying and by establishing a limit for residual solvent. The tentative designation was removed.

At its present meeting, the Committee considered a submission that comprised a review of all the available information, including studies completed after the sixty-eighth meeting and some older studies not highlighted in the previous reviews by the Committee. The new studies included four toxicological studies with rebaudioside A in experimental animals and clinical trials on the effects of steviol glycosides on blood pressure in healthy volunteers with normal or low-normal blood pressure and on glucose homeostasis in men and women with type 2 diabetes mellitus. Additionally, a literature search was carried out to identify studies published since the sixty-eighth meeting.

Toxicological data

The toxicokinetic studies confirmed that intact stevioside and rebaudioside A are poorly absorbed, but they are hydrolysed by the intestinal microflora to steviol, which is well absorbed. After absorption, steviol is metabolized mainly to steviol glucuronide, which is excreted in the urine of humans. In rats, steviol glucuronide is excreted in the bile and deconjugated in the lower intestine, before elimination as steviol in the faeces. Pharmacokinetic parameters indicate that systemic exposure to steviol is greater after administration of stevioside than after administration of rebaudioside A in rats, whereas systemic exposure in humans is primarily to steviol glucuronide and is similar for stevioside and rebaudioside A.

The older studies identified in the submission mainly involved material of unknown composition or not meeting the present specification and were not informative for the evaluation. The results of the new studies in animals were consistent with the results of previous studies. In two new 13-week studies in rats fed diets containing rebaudioside A, no adverse effects were observed at dietary concentrations of up to 36 000 mg/kg and up to 50 000 mg/kg, respectively. The latter concentration is considered to be a NOEL, equal to doses of 4161 mg/kg bw per day in males and 4645 mg/kg bw per day in females, expressed as rebaudioside A (1370 mg/kg bw per day in males and 1530 mg/kg bw per day in females, expressed as steviol).

At its fifty-first meeting, the Committee reviewed a number of studies of reproductive and developmental toxicity with stevioside and *Stevia* extracts and noted that administration of stevioside (purity 90–96%) at doses of up to 2500 mg/kg bw per day in hamsters and 3000 mg/kg bw per day in rats had no effect. The Committee also noted that, although an aqueous infusion of *S. rebaudiana* administered orally to female rats was reported to cause a severe, long-lasting reduction in fertility, the contraceptive effect of *Stevia* was probably not due to stevioside. Stevioside (purity 95.6%) had neither teratogenic nor embryotoxic effects at doses of up to 1000 mg/kg bw per day in rats treated by gavage. The multigeneration study of reproductive toxicity reviewed at the present meeting did not reveal adverse effects with rebaudioside A at the highest dose tested, 2048–4066 mg/kg bw per day (674–1339 mg/kg bw per day expressed as steviol). This supports the previous conclusion of the Committee that administration of steviol glycosides was unlikely to be associated with adverse reproductive effects.

The new studies in humans were designed to address the issues that the Committee raised at its sixty-third meeting concerning evidence to demonstrate that the putative pharmacological effects of steviol glycosides would not be found at the exposure levels resulting from the proposed use as a food additive.

Steviol glycosides did not have adverse effects on diabetic control or on blood pressure in patients with type 2 diabetes given 1000 mg of rebaudioside A per day (mean dose of rebaudioside A, 10.2 mg/kg bw per day, equivalent to 3.4 mg/kg bw per day expressed as steviol) for 16 weeks. No studies were conducted in patients with type 1 diabetes. However, the Committee at its present meeting noted that the purported mechanism of action of steviol glycosides on glucose homeostasis involves enhanced secretion of insulin from the pancreas when there is impaired response to glucose stimulation. In contrast, type 1 diabetes is characterized by a permanent inability of the pancreatic β-cell to produce insulin, and therefore effects of steviol glycosides were considered unlikely in this subgroup.

No clinically significant changes in blood pressure parameters were seen in normotensive individuals or in a subset of these individuals with blood pressure below the median who took rebaudioside A at a dose of 1000 mg/day (mean dose of rebaudioside A, 14 mg/kg bw per day, or 4.6 mg/kg bw per day expressed as steviol) for 4 weeks.

Assessment of dietary exposure

The Committee evaluated information on dietary exposure to steviol glycosides from its sixty-third meeting and additional information concerning potential dietary exposure to rebaudioside A submitted by a sponsor. All the exposure results are presented in terms of equivalents of steviol, based on a conversion of 40% from the steviol glycoside, stevioside (relative molecular mass: steviol, 318; stevioside, 805), or 33% from rebaudioside A (relative molecular mass 967).

The Committee used the GEMS/Food database to prepare updated international estimates of dietary exposure to steviol glycosides (as steviol). It was assumed that steviol glycosides would replace all dietary sugars at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, 200:1. The dietary exposures ranged from 0.9 mg/kg bw per day (cluster J) to 5 mg/kg bw per day (clusters B and M). The Committee evaluated estimates of dietary exposure per capita derived from disappearance (poundage) data supplied by Japan and China.

The Committee evaluated an estimate of dietary exposure to steviol glycosides based on the replacement of all dietary sugars in the USA. Using a per capita estimate of 176 g of caloric sweetener per day, the Committee calculated that the consumption of steviol glycosides would be 5.8 mg/kg bw per day. Additionally, published estimates of exposure to rebaudioside A, based on exposure to other high-intensity sweeteners and using the principle of equivalent sweetness, were evaluated by the Committee. These estimates were 1.5 mg/kg bw per day for diabetic children and adults and

1.7 mg/kg bw per day for non-diabetic children consuming the sweetener at a high percentile of the exposure distribution, taken to be greater than the 90th percentile.

Table 4 summarizes the exposures to steviol glycosides (as steviol) evaluated or derived by the sixty-third and current meetings of the Committee.

Table 4
Estimates of dietary exposure to steviol glycosides, expressed as steviol

Estimate	Exposure (mg/kg bw per day)
GEMS/Food (per capita)	0.9–5.0
Japan (per capita disappearance)	0.04
Japan (per capita replacement estimate)	3
USA (per capita replacement estimate)	5.8
Diabetic adult (high-percentile estimate)	1.5
Diabetic child (high-percentile estimate)	1.5
Non-diabetic child (high-percentile estimate)	1.7

The Committee concluded that the replacement estimates were highly conservative and that dietary exposure to steviol glycosides (as steviol) would likely be 20–30% of these values. The published estimates based on equivalent sweetness were taken as more representative of probable dietary exposure at a high percentile of the exposure distribution.

Evaluation

From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg/kg bw per day was identified. At its sixty-third meeting, the Committee set a temporary ADI of 0-2 mg/kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL[1] for stevioside of 970 mg/kg bw per day (383 mg/kg bw per day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5–25.0 mg/kg bw per day (5–10 mg/kg bw per day expressed as steviol).

The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or

[1] Before the sixty-eighth meeting, the Committee used the term NOEL to include the current definitions of both NOEL and NOAEL. According to the decision taken by the Committee at its sixty-eighth meeting (Annex 1, reference *187*), this NOEL would now be termed a NOAEL.

low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0– 4 mg/kg bw expressed as steviol.

The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

The existing specifications were revised.

An addendum to the toxicological monograph was prepared.

3.1.9 *Sulfites: assessment of dietary exposure*

Explanation

Dietary exposure to sulfites was evaluated by the Committee at its present meeting at the request of CCFA at its Thirty-ninth Session (*4*). The Committee was asked to consider all data necessary for the assessment of dietary exposure from all foods, including use levels, owing to concern that the ADI might be exceeded.

Sulfites have a number of technological functions, including antioxidant, bleaching agent, flour treatment agent and preservative, and are used in a wide variety of applications in the food industry. The terms “sulfites” and “sulfiting agents” usually refer to the gas sulfur dioxide and sodium, potassium and calcium sulfites, hydrogen sulfites and metabisulfites. Throughout section 3.1.9 of the present report, the concentration of sulfites in food is expressed as sulfur dioxide. The additives listed under sulfites in the current Codex GSFA are sulfur dioxide (International Numbering System [INS] 220), sodium sulfite (INS 221), sodium hydrogen sulfite (INS 222), sodium metabisulfite (INS 223), potassium metabisulfite (INS 224), potassium sulfite (INS 225), calcium hydrogen sulfite (INS 227), potassium bisulfite (INS 228) and sodium thiosulfate (INS 539).

Sulfur dioxide and sulfites were evaluated by the Committee at its sixth, eighth, ninth, seventeenth, twenty-seventh, thirtieth and fifty-first meetings (Annex 1, references *6*, *8*, *11*, *32*, *62*, *73* and *137*). At its seventeenth meeting, the Committee established an group ADI for sulfites of 0–0.7 mg/kg bw, expressed as sulfur dioxide.

In its last evaluation of sulfites at the fifty-first meeting, the Committee noted that potential dietary exposures based on maximum levels (MLs)[1] proposed in the draft Codex Alimentarius Commission GSFA and on national mean food consumption data exceeded the ADI in the three Member States that submitted such data. Six Member States also submitted data in which dietary exposure was assessed on the basis of MLs in their national regulations and on mean food consumptions; mean potential dietary exposure of consumers of food containing sulfites did not exceed the ADI. The potential for high-percentile consumers of foods containing sulfites to exceed the ADI was shown to exist, but available data were insufficient to estimate the number of such consumers or the magnitude and duration of intake greater than the ADI.

As sulfites are known to cause adverse reactions in specific subsets of the population, there are specific provisions in the Codex General Standard for the Labelling of Prepackaged Food (*12*) and in national legislation in a number of countries for the labelling of foods and beverages containing sulfites. This issue and that of the potential acute toxicity of sulfites in general were not dealt with by the Committee at its present meeting, which focused on long-term dietary exposure, in line with the CCFA request.

Analytical methods

Several methods are available for the determination of sulfites in foods and beverages, the choice of method depending, among other factors, on the matrix to be analysed and the expected concentration of sulfites. Published methods are generally based on the known chemistry and reactivity of sulfites with the matrix and require some means of recovering sulfur dioxide. They fall into two basic categories: methods that require an initial distillation of the test sample to free the sulfur dioxide, and those that use a non-distillation reaction to achieve the same end-point. The combination with organic constituents, the equilibrium between the various inorganic forms, the volatilization of sulfur dioxide and the oxidation to sulfates are all important reactions, and their relative importance will depend mostly on the food involved.

The most common methods involve distillation of sulfur dioxide from a highly acidified sample, followed by titration, colorimetric, polarographic or ion chromatographic determination. The Monier-Williams method has been the procedure most widely used for the determination of sulfite in foods and beverages and has been adopted as the official method in various countries. Today, many modifications of the Monier-Williams method exist for application to particular matrices. The AOAC International Official Methods of Analysis 962.16 and 990.28, for example, are applicable to foods and

[1] Throughout section 3.1.9 of the present report, the term maximum level (ML) is used to represent any maximum level set for a regulatory purpose at a national or international level.

beverages in the presence of other volatile compounds, but are not applicable to dried onions, leeks and cabbages. The reference method adopted by the International Organisation of Vine and Wine to measure free and total sulfites in wine is also a modification of the Monier-Williams procedure.

Alternative methods include enzymatic, liquid chromatographic, differential pulse polarographic, capillary electrophoretic and flow injection techniques coupled with spectrophotometry, amperometry, potentiometry and chemiluminescence.

Current status of sulfites in Codex and national legislation

Owing to their multiple functions, sulfites are listed for use in a wide variety of solid and liquid foods in the current GSFA. Most provisions for solid foods are in the range of 15– 500 mg/kg. Most provisions for liquid foods are in the range of 50–200 mg/kg.

Examples of solid foods for which there are provisions in the current Codex GSFA are processed vegetables (up to 500 mg/kg), processed fish and seafood (up to 150 mg/kg) and processed fruit (up to 1000 mg/kg in dried fruit). Examples of liquid foods for which there are provisions are alcoholic drinks, including beer (up to 50 mg/l) and wine (up to 350 mg/l), fruit and vegetable juices (up to 50 mg/l) and water-based flavoured drinks (up to 70 mg/l).

For sulfites, CCFA adopted MLs that are lower than the draft MLs that were used for the assessment of dietary exposure performed by the Committee at its fifty-first meeting. For example, for the category 04.1.2.2 “dried fruit”, the draft ML was 5000 mg/kg, and the ML in the current Codex GSFA is 1000 mg/kg.

In most national legislation that regulates the use of sulfites, there are provisions for the same solid and liquid foods as in the current GSFA. The MLs set in some national regulations are higher than the MLs in the current GSFA for a number of categories of foods and beverages. This is the case for dried fruit (2000 mg/kg in the EU and Republic of Korea and 3000 mg/kg in Australia and New Zealand versus 1000 mg/kg in the current GSFA), dried vegetables (3000 mg/kg in Australia and New Zealand versus 500 mg/kg in the current GSFA) and lemon juice (350 mg/l in the EU versus 50 mg/l in the current GSFA).

The Committee noted that there are no provisions for the use of sulfites for any meat product either in the current GSFA or in Codex commodity standards. On the other hand, there are provisions for processed meats in some national legislation made available to the Committee. Sulfites are known to destroy thiamine. For this reason, the use of sulfites in foods that are

considered an important source of thiamine, such as meat products, is not permitted in some countries (e.g. Brazil) or is permitted only for limited applications (e.g. in the EU, sulfites can be used only in breakfast sausages and burger meats with vegetables and/or cereals, at a ML of 450 mg/kg, but cannot be used in burger meat in general). In Australia and New Zealand, sulfites can be used at up to 500 mg/kg in broader categories: in burger meat in general and in all sausages containing raw meat.

Data made available to the Committee

Data were made available to the Committee for the present evaluation through submissions by Australia, Brazil, Germany and the USA. The Comité Européen des Fabricants de Sucre sent general comments on the sugar-related provisions in the current GSFA, but without any specific information in relation to dietary exposure. The data presented in these submissions were complemented with data from the literature referring to France, Italy, Lebanon and the United Kingdom. Only data published since the last evaluation of sulfites by the Committee in 2000 were considered in the present evaluation. Therefore, the data presented in the submission by the USA, which predated 2000, were not considered.

The data made available to the Committee comprised data on the concentration of sulfites in foods and beverages and on dietary exposure assessments based on model diets, individual food surveys and a total diet study (TDS).

Concentration of sulfites in foods and beverages

Information on the concentration of sulfites in foods and beverages present on the market and, where applicable, after cooking is very useful to complement the information on MLs in Codex Alimentarius Commission and national legislation, since it allows the assessment of current levels of exposure rather than potential levels of exposure in the population. In fact, as for other additives, sulfites may not be used in all items for which there are provisions and could be used at levels differing from the MLs. Concentrations of sulfites were shown to be reduced during storage (with observed reductions ranging from 25% to 50% after 1 month of storage for fish products, potatoes and dried fruit) and during cooking (with observed reductions of about 40% in cooked burgers, reduction of 70% in Thai noodles and reduction to non-detectable levels in dried mushrooms and peeled potatoes in brine).

Germany submitted analytical data on the concentration of sulfites in a wide range of foods and beverages present on the German market, and Brazil provided such data for wine and fruit juice. Further analytical data referred to foods and beverages present on the markets of the United Kingdom (only for soft drinks and minced meat) and Italy. In the case of Australia, Italy and

Lebanon, analytical determinations in foods were performed after cooking. Data on occurrence/use levels of sulfites in foods available on the French market, as reported by the food industry based on their product recipes, were also made available to the Committee, together with analytical determinations in wine.

The analytical determinations and reported occurrences suggest that in all these countries, which belong to different regions of the world, sulfites are frequently added in many of the categories of foods and beverages for which there are provisions in the current GSFA.

The analytical data on wine in Brazil, France, Germany and Italy showed that the average concentration of sulfites may vary according to the country and the type of wine, but all were in the range of 70–130 mg/l — i.e. they are lower than the provisions in current GSFA (350 mg/l) or national legislation. Two studies showed that the current average levels of residue are lower than those found in previous decades. A limited number of single samples exceed the MLs, reaching more than 1000 mg/l.

The analytical data in other foods and beverages show that, in line with MLs set by national legislation being higher than those set by the Codex Alimentarius Commission, mean concentrations of sulfites can be greater than the MLs of the current GSFA. This is the case for some non-alcoholic beverages and for dried fruit. Thus, in Brazil, the mean concentration of sulfites in one type of fruit juice was greater than the ML for fruit juices in the current GSFA. The same was true for lemon and lime juices and for barley water in the United Kingdom. In Australia, the mean concentration of sulfites in dried fruit ranged from 1200 to 2000 mg/kg, whereas the ML in the current GSFA is 1000 mg/kg.

In Australia, sulfites are largely used in sausages containing raw beef and in burger meat, with average concentrations in the range of 100–300 mg/kg.

The observed mean concentrations of sulfites in some food categories were found to be close to the national MLs, and concentrations of sulfites in single samples occasionally exceeded the national MLs. This was shown to occur for fruit juice, dried fruit, potato-based snacks, mustard and fine bakery wares. Mean concentrations that were greater than the national MLs were identified in some food categories, suggesting either more frequent or more significant excesses. This was the case for dried tomatoes and horseradish in Germany and for fruit fingers in Australia. In Lebanon, the mean concentration of sulfites was in excess of the ML in the current GSFA for biscuits and crackers. Some analytical data provided to the Committee were related to the illegal use of sulfites in minced meat in Scotland and Australia. Sulfites are not authorized for this use in the current national legislation of these countries (although they were authorized in Scotland until 1977).

Budget method

The budget method is generally used as a screening method at the first step of the assessment of dietary exposure in the evaluations of food additives performed by the Committee. It is used to identify the need for a refined assessment of dietary exposure. In the present evaluation, the budget method was not applied, since a refined assessment of dietary exposure had been requested by the CCFA.

Assessment of long-term dietary exposure based on model diets

Brazil submitted estimates of dietary exposure prepared by combining the average concentration of sulfites in red wine (70 mg/l) and white wine (122 mg/l) present on its market with three hypothetical scenarios of regular consumption of wine: 150, 300 or 450 ml/day. Considering a standard body weight of 60 kg, dietary exposure would cover between 29% and 71% of the ADI for red wine and between 43% and 129% of the ADI for white wine. The Committee noted that, on the basis of provisions for wine in the current GSFA (350 mg/l), the same exposure scenarios would range from 125% to 375% of the ADI.

The Committee further noted that the daily consumption of 450 ml of wine is not an unrealistic scenario, since it corresponds to the observed high-percentile consumption of wine in countries where it is regularly consumed: the 95th percentile of consumption in a 7-day nationwide survey in Italy was 450 ml/day, and the 97.5th percentile of consumption in a 7-day nationwide survey in France was 600 ml/day.

A model diet was developed for Italy by combining selected foods and beverages in order to design realistic meals with the highest possible dietary exposure to sulfites, while being based on a regular food pattern and on standard portions and recipes for a child of 30 kg and an adult of 60 kg. The sulfite contents of different meals designed for children and adults were calculated using both EU MLs and average concentrations determined analytically. The total dietary exposures from different selections of meals (one breakfast plus two main meals plus two between meals) were calculated. Considering the EU MLs, a daily dietary exposure of 68 mg could be reached by children, corresponding to 325% of the ADI; and a daily dietary exposure of 123 mg could be reached by adults, corresponding to 294% of the ADI.

The results obtained by using the mean concentration in ready-to-consume foods and recipes were lower than those obtained by using the MLs and would reach 111% of the ADI in children and 120% of the ADI in adults. In children, the main contributor was dried fruit contained in muesli (contributing to 43% of the ADI, based on daily consumption of 50 g muesli containing sulfites at

180 mg/kg). In adults, the main contributors were wine (contributing to 44% of the ADI based on the daily consumption of 200 ml of wine containing sulfites at 92 mg/l) and peanuts (contributing to 15% of the ADI based on the daily consumption of 15 g of peanuts containing sulfites at 385 mg/kg). Other food items were shown to contribute at least 10% of the ADI: beer in adults and, for children, soft drinks, mashed potatoes and mustard. All these products would be significant sources of sulfites in the general population, with the exception of the soft drinks, since in Italy only very specific products (for bulk dispensers) would contain sulfites.

Assessment of long-term dietary exposure based on a total diet study

A TDS was performed in Australia for young girls and boys (aged 2–5 years), schoolgirls and schoolboys (aged 6–12 years), teenage girls and boys (aged 13–18 years) and adult women and men (aged 19 years and older) based on a 1-day 24-h recall survey of 13 858 subjects. All the foods examined in the study were prepared as ready to be consumed before analysis. Overall, 90% of the respondents were consumers of foods or beverages containing sulfites.

The mean estimated dietary exposures for consumers ranged from 14% of the ADI for teenage girls to 71% of the ADI for young boys. Mean estimated dietary exposure for consumers aged 2 years and older was 29% and 35% of the ADI for both males and females.

The 95th percentile of estimated dietary exposures in consumers exceeded the ADI for most population groups considered, ranging from 86% of the ADI for teenage girls to 271% of the ADI for young boys. The 95th percentile of estimated dietary exposure for consumers aged 2 years and older, which could be used to represent the lifetime exposure of high consumers, was approximately 130% of the ADI for males and females.

The major contributors to the mean total dietary exposure to sulfites in consumers differed between children and adults. In young children, schoolchildren and teenagers, the three main contributors to mean dietary exposure were beef sausages (contributing up to 20% of the ADI, according to age and sex), dried apricots (up to 20%) and cordial (up to 15%). In adults, the main contributors to mean dietary exposure were white wine (up to 12%), beef sausages (up to 7%) and dried apricots (up to 6%).

Assessment of long-term dietary exposure based on individual dietary surveys

An assessment of dietary exposure to sulfites based on individual dietary surveys was submitted by Brazil. Additional information from France and Lebanon was made available to the Committee. The assumptions made and the results of these assessments are summarized in Table 5.

Table 5
Assessment of dietary exposure to sulfites based on individual dietary surveys

Country	Type of survey (year)	Assumptions used for the assessment of dietary exposure	Indicator	Dietary exposure to sulfites (mg sulfur dioxide/kg bw per day)	% ADI
Brazil	One 24-h recall; adolescents aged 11–17 years; sample size 140 (2002)	Assessment of dietary exposure only from fruit juice Mean analytical data in selected fruit juices (containing sulfites according to the label) Adjusted for individual body weight Corrections for dilution according to instructions from manufacturers in case of concentrates	Mean (consumers only) 90th percentile (consumers only) 95th percentile (consumers only) 97.5th percentile (consumers only)	0.11–0.41[a] 0.19–0.70[a] 0.24–0.88[a] 0.30–1.09[a]	16–59 27–100 34–125 43–155
France	7-day diary record; aged 3 years and older; sample size 2492 (1998–1999)	Average use levels (reported data from food industry) Adjusted for individual body weight Two different scenarios related to the food habits: 1) consumers randomly consume foods that do or do not contain sulfites; 2) consumers always consume foods that contain sulfites	*First scenario:* Mean (adults) Mean (children aged 3–14 years) 97.5th percentile (adults) 97.5th percentile (children aged 3–14 years) *Second scenario:* Mean (adults) Mean (children aged 3–14 years) 97.5th percentile (adults) 97.5th percentile (children aged 3–14 years)	 0.2 0.1 0.83 0.32 0.27 0.25 1.1 0.9	 29 14 119 46 39 36 157 129

Lebanon	One 24-h recall; children aged 9–13 years and adolescents aged 14–18 years; sample size 230 (2002–2003)	Mean concentration (analytical results) Foods analysed as “ready to consume” Adjusted for individual body weight	Mean (all population) Mean (consumers only) 95th percentile (consumers only)	0.4 0.44 1.5	57 63 214

[a] The range represents the minimum and maximum level of dietary exposure to sulfites depending on the type of fruit juice.

In Brazil, high potential dietary exposure to sulfites from fruit juices in teenagers was assessed by combining the consumption of any fruit juice as reported in a 24-h dietary recall with the average concentrations of sulfite determined by chemical analysis in those fruit juices containing added sulfites (according to the label). Dietary exposure was expressed in relation to individual body weights in consumers only (140 subjects). Mean dietary exposure from fruit juices would vary from 16% to 59% of the ADI, and dietary exposure at the 97.5th percentile would vary from 43% to 155% of the ADI. The results indicate that teenagers who are regular high consumers of specific fruit juices containing sulfites are potentially at risk of exceeding the ADI and that in mean consumers, fruit juices containing sulfites cover a significant portion of the ADI. The Committee noted that, owing to the small size of the Brazilian sample, the high percentiles that were assessed bear significant uncertainty and the true high percentile of dietary exposure could be higher or lower.

In France, dietary exposure to sulfites was estimated by combining national individual consumption data with the occurrence/use level of sulfites as reported by the food industry, based on two different scenarios. In the first scenario, the mean concentration of sulfites in each food group was considered, including "zero" values (overall, 45% of the products considered were reported to contain no sulfites). This scenario is aimed at representing consumers who randomly consume foods that do or do not contain food additives. In the second scenario, the "zero" values were eliminated, thus assuming that consumers always consumed foods that contained sulfites. This conservative scenario simulates the situation of brand loyalty. In both scenarios, the potential dietary exposure to sulfites was higher among adults than among children owing to the consumption of wine.

In the first scenario, the potential dietary exposure to sulfites in adults did not exceed the ADI at the mean but did at the 97.5th percentile (0.83 mg/kg bw per day). The major contributor to mean dietary exposure in adults was wine, covering 20% of the ADI. In children, dietary exposure was within the ADI (0.3 mg/kg bw per day) at the 97.5th percentile.

In the second scenario, dietary exposure at the 97.5th percentile was higher than in the first scenario and exceeded the ADI in adults (1.1 mg/kg bw per day) and children (0.9 mg/kg bw per day). In the adults, wine was the main contributor to mean and high dietary exposure (covering 20% of the ADI at the mean), followed by dried fruits (covering about 5% of the ADI at the mean). Processed potatoes, peeled potatoes and dried fruits were the main contributors to mean and high dietary exposure in children (with each of these food groups covering about 10% of the ADI at the mean).

In Lebanon, dietary exposures to sulfites were calculated by combining the consumption data for selected foods in 230 children and adolescents with the

mean analytical residue levels in the food as consumed, after cooking. The mean dietary exposure to sulfites was within the ADI (61%), whereas it was greater than the ADI (214%) at the 95th percentile. The major contributor was nuts, covering 178% of the ADI in high consumers. Overall, dietary exposure was greater than the ADI in 10% of subjects. The Committee noted that, owing to the small size of the Lebanese sample, the high percentiles that were assessed bear significant uncertainty and that the true high percentile of dietary exposure could be higher or lower.

Evaluation of long-term dietary exposure to sulfites and recommendations

In its previous evaluation of sulfites in 2000, the Committee noted that the use of sulfites at the MLs proposed in the draft Codex Alimentarius Commission GSFA would lead to dietary exposure in excess of the ADI. As a result, the MLs for sulfites are now lower in the current version of the Codex GSFA. However, a number of national governments have not yet reacted to the new MLs.

In the present evaluation, dietary exposure was assessed in a number of Member States based on analytical determinations of products on the market and in ready-to-consume foods after cooking. Sulfite residues were sometimes found to be greater than the national MLs and the MLs of the current Codex GSFA — in particular for non-alcoholic beverages and dried fruit.

In all countries for which data were available, the total dietary exposure to sulfites in the general population was within the ADI at the mean but greater than the ADI at high percentiles of exposure. In particular, dietary exposure was shown to reach twice the ADI in children and teenagers in some countries. This was true even when the concentration of sulfites in ready-to-consume foods was taken into consideration. The Committee noted that some of the assessments of dietary exposure were derived from 1-day food consumption survey data, which are known to overestimate long-term consumption for rarely consumed foods. For this reason, the Committee investigated whether the main contributors to dietary exposure were foods and beverages that are commonly consumed.

The Committee noted that the main contributors to total dietary exposure to sulfites differ in the different countries owing to differing patterns of consumption of foods to which sulfites may be added and to differing patterns of use of sulfites in foods. Thus, dried fruit, sausages and non-alcoholic beverages were the main contributors of sulfites in some countries, whereas these foods are generally produced without the use of sulfites in other countries.

In children and teenagers, a significant contribution to mean exposure to sulfites could come from fruit juices and soft drinks (including cordial), sausages, various forms of processed potatoes, dried fruit and nuts.

In all countries where wine is regularly consumed, it was one of the main contributors to exposure in adults. Dietary exposure in high consumers of wine was shown to exceed the ADI based on MLs in the Codex GSFA, MLs in national legislation or the average concentration determined analytically (about 100 mg/l). Other significant contributions to dietary exposure in the adult population come from dried fruit, sausages and beer.

Countries that have not yet done so could consider collecting data on the current use of sulfites in foods and beverages available on their markets and investigating whether dietary exposure in some subpopulations exceeds the ADI. On the basis of this investigation, individual countries and the food industry could consider the possibility of taking one or more of the following measures to reduce dietary exposure to sulfites so that the ADI is not exceeded in the population: 1) align national legislation with Codex MLs where these are lower; 2) take action to effectively enforce national MLs; 3) encourage research on alternative methods of preservation, particularly on applications in which the use of sulfites is responsible for a significant contribution; or 4) take action so that the use of sulfites is reduced in foods where safe alternative solutions are available.

Codex codes of practice for certain groups of food commodities, such as fruit juice, dried fruit and processed meat, could include suggestions to help countries and the food industry in the implementation of a reduction of the use of sulfites in food.

A monograph was prepared.

3.2 Revision of specifications

3.2.1 *Canthaxanthin*

The Committee was made aware that in the specifications for canthaxanthin, the wording of the criterion for the assay could be misinterpreted. The Committee decided to change the original text "Not less than 96% of total colouring matters (expressed as canthaxanthin)" in the electronic version of the specifications on the FAO JECFA website to read "Not less than 96% total colouring matters (expressed as canthaxanthin)". It was also decided that it was unnecessary to republish the print version.

3.2.2 *Carob bean gum and carob bean gum (clarified)*

The Committee was requested by the Codex Committee on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session (*13*) to review the specifications monograph entitled "Carob bean gum" and noted that two grades of product were covered. At its sixty-seventh meeting (Annex 1,

reference *184*), the Committee decided to prepare two specifications monographs, "Carob bean gum" and "Carob bean gum (clarified)". The specifications in both monographs were designated tentative, and further information was requested on the characterization of these products and the analytical procedures for the residual solvents. At the present meeting, the Committee revised the specifications and decided that the information received was sufficient to remove the tentative designations.

3.2.3 *Chlorophyllin copper complexes, sodium and potassium salts*

The Committee was informed that the Colour Index (C.I.) International number in the specifications for chlorophyllin copper complexes, sodium and potassium salts was incorrectly stated. The Committee decided to include the correct number, C.I. No. 75815, in the electronic version of the specifications on the FAO JECFA website. It was also decided that it was unnecessary to republish the print version.

3.2.4 *Fast Green FCF*

The Committee was informed that an error had been introduced into the specification for Fast Green FCF published in the Combined Compendium of Food Additive Specifications (Annex 1, reference *180*) when the text from FAO Food and Nutrition Paper 52 was transcribed. The value for absorptivity in the determination of the quantity of leuco base was corrected to read 0.156 in the electronic version of the specifications on the FAO JECFA website. It was also decided that it was unnecessary to republish the print version.

3.2.5 *Guar gum and guar gum (clarified)*

The Committee was requested by CCFAC at its Thirty-seventh Session (*13*) to review the specifications monograph entitled "Guar gum" and noted that two grades of product were covered. At its sixty-seventh meeting (Annex 1, reference *184*), the Committee decided to prepare two specifications monographs, "Guar gum" and "Guar gum (clarified)". The specifications in both monographs were designated tentative, and further information was requested on the characterization of these products and the analytical procedures for the residual solvents. At its present meeting, the Committee revised the specifications and decided that the information received was sufficient to remove the tentative designations.

3.2.6 *Iron oxides*

The Committee at its sixty-third meeting (Annex 1, reference *173*) prepared specifications for iron oxides and included a maximum limit of 1.0% for water-soluble matter. The Committee at its present meeting recognized that

a test method for water-soluble matter was required. A test method was included, and the specifications were revised.

3.2.7 *Isomalt*

The Committee was informed that the method for determination of nickel in the specifications for isomalt was incomplete. The method, "Determination of nickel in polyols", described in Volume 4 of the Combined Compendium of Food Additive Specifications (Annex 1, reference *180*) was similarly incomplete and required revision. The method was revised and will be published in the Compendium of Food Additive Specifications, FAO JECFA Monographs 5 (Annex 1, reference *192*). The incomplete method in the specifications for isomalt was replaced by a reference to the revised method.

3.2.8 *Monomagnesium phosphate*

Monomagnesium phosphate was placed on the agenda of the present meeting at the request of CCFA at its Thirty-ninth Session (*4*) to reinstate the specifications that had been withdrawn by the Committee at its sixty-fifth meeting (Annex 1, reference *178*). The Committee at its sixty-first meeting (Annex 1, reference *166*) had revised the existing tentative specifications but maintained them as tentative with a second request for information on the maximum limits and methods for loss on drying and loss on ignition for the dihydrate. The Committee had noted that the tentative specifications would be withdrawn unless the requested information was received before the end of 2004 and did so at its sixty-fifth meeting, as no additional information was received. The Committee at its present meeting received additional information on the levels and methods for loss on drying and loss on ignition for the dihydrate.

The previously withdrawn tentative specifications were reinstated and revised, and the tentative designation was removed.

3.2.9 *Patent Blue V*

Patent Blue V was placed on the agenda of the present meeting to obtain missing information on the tests for its leuco base. The Committee obtained the required information and revised the specifications accordingly, including minor editing.

3.2.10 *Sunset Yellow FCF*

Sunset Yellow FCF was placed on the agenda of the present meeting for revision of specifications at the request of CCFA at its Thirty-ninth Session (*4*). Specifically, CCFA requested addition of an analytical method and

maximum limit for Sudan I (1-(phenylazo)-2-naphthalenol), a known impurity in Sunset Yellow FCF. Sudan I has been shown to be genotoxic and carcinogenic in experimental studies. Therefore, the levels of Sudan I in Sunset Yellow FCF should be as low as practicably possible.

A validated analytical method for Sudan I in Sunset Yellow FCF has recently been published in the *Journal of AOAC International* (vol. 90, no. 5, 2007, pp. 1373–1378). Using reversed-phase liquid chromatography, Sudan I can be directly determined in Sunset Yellow FCF. The limit of determination for this method is 0.4 mg/kg. In setting the maximum limit of Sudan I in Sunset Yellow FCF, the Committee took into account a survey over a 3-year period, reported in the journal article, to determine Sudan I in 28 samples of Sunset Yellow FCF. These samples were obtained from 17 international manufacturers. The Committee observed that, of the 28 samples, 16 samples (57%) were below the limit of determination of 0.4 mg/kg, 5 samples (18%) showed residues of Sudan I ranging from 0.5 to 1 mg/kg and only 7 samples (25%) showed residues greater than 1 mg/kg. Therefore, 75% of the samples contained Sudan I at concentrations equal to or less than 1 mg/kg. Based on these data, the Committee established a maximum limit for Sudan I of 1 mg/kg in Sunset Yellow FCF.

The Committee revised the existing specifications.

No toxicological assessment was performed.

3.2.11 *Trisodium diphosphate*

Trisodium diphosphate was placed on the agenda of the present meeting at the request of CCFA at its Thirty-ninth Session (*4*) to reinstate the specifications that had been withdrawn by the Committee at its sixty-fifth meeting (Annex 1, reference *178*). The Committee at its sixty-first meeting (Annex 1, reference *166*) had revised the existing tentative specifications but maintained them as tentative with a second request for information on the maximum limit and method for loss on drying for the monohydrate. The Committee had noted that the tentative specifications would be withdrawn unless the requested information was received before the end of 2004 and did so at its sixty-fifth meeting, as no additional information was received. The Committee at its present meeting received additional information on the loss on drying for the monohydrate.

The previously withdrawn tentative specifications were reinstated and revised, and the tentative designation was removed.

4. Flavouring agents

4.1 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

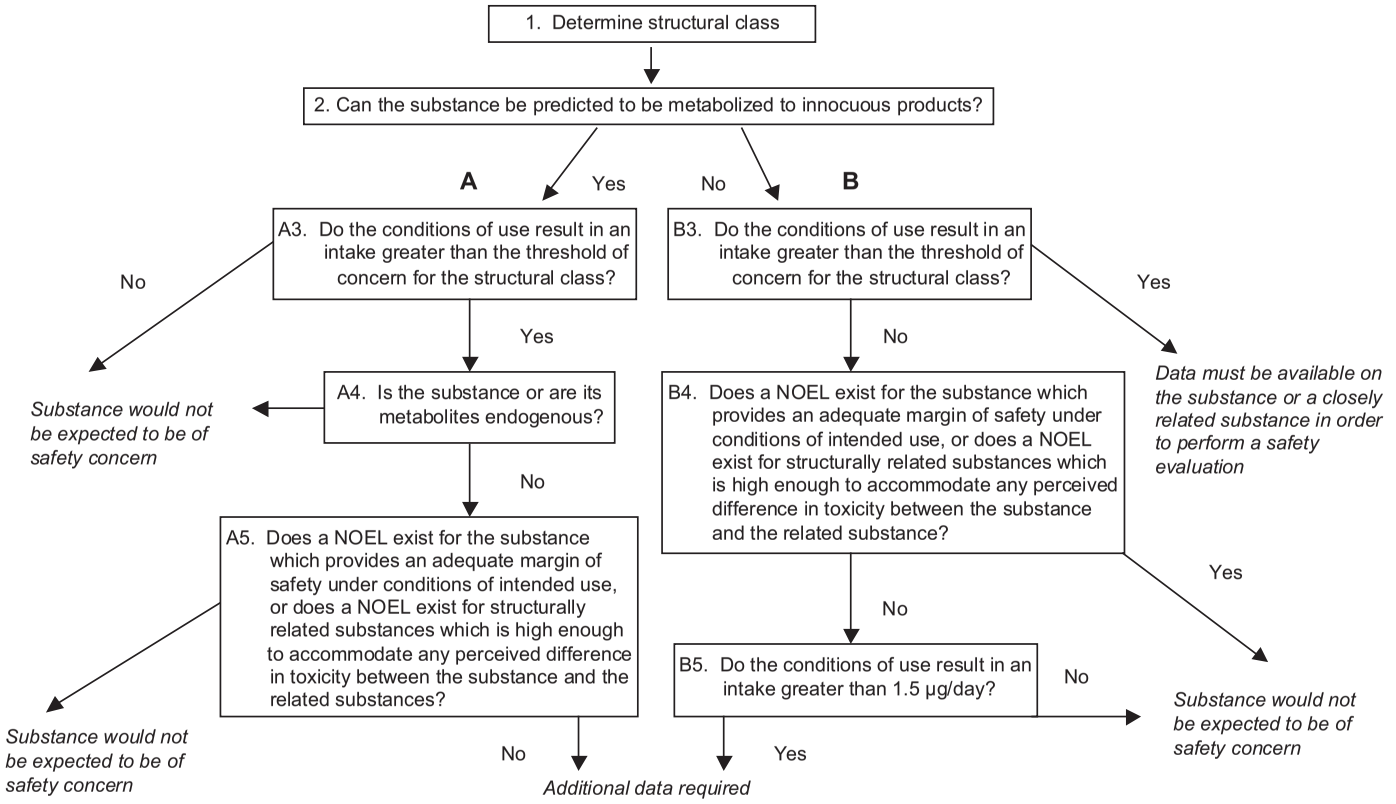
Ten groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154*, *160*, *166*, *173* and *178*). In applying the Procedure, the chemical is first assigned to a structural class as identified by the Committee at its forty-sixth meeting (Annex 1, reference *122*). The structural classes are as follows:

- *Class I.* Flavouring agents that have simple chemical structures and efficient modes of metabolism that would suggest a low order of toxicity by the oral route.
- *Class II.* Flavouring agents that have structural features that are less innocuous than those of substances in class I but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- *Class III.* Flavouring agents that have structural features that permit no strong initial presumption of safety or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations, the Committee used the following definitions, adapted from the report of its forty-sixth meeting (Annex 1, reference *122*):

- *Innocuous metabolic products* are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.
- *Endogenous substances* are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated intake of a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

Figure 1 **Procedure for the Safety Evaluation of Flavouring Agents**



Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data were derived from surveys in Europe, Japan and the USA. In Europe, a survey was conducted in 1995 by IOFI, in which flavour manufacturers reported the total amount of each flavouring agent incorporated into food sold in the EU during the previous year.

Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products.

In the USA, a series of surveys was conducted between 1970 and 1987 by the NRC of the NAS (under contract to the FDA) in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximum levels at which each substance was added in a number of broad food categories.

In using the data from these surveys to estimate intakes of flavouring agents, it was previously assumed that only 60% of the total amount used is reported in the USA and 80% of the amount used is reported in Europe and that the total amount used in food is consumed by only 10% of the population. At the present meeting, a correction factor of 0.8 was applied to the annual production volumes reported in the recent surveys from Europe, Japan and the USA (*14*, *15*, *16*).

$$\text{Intake (µg/person per day)} = \frac{\text{annual volume of production (kg)} \times 10^9 \text{ (µg/kg)}}{\text{population of consumers} \times 0.6 \text{ (or } 0.8) \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe, 13×10^6 in Japan and 28×10^6 in the USA.

4.1.1 *Aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters: additional compounds*

The Committee evaluated a group of flavouring agents consisting of 20 aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters. This group included 2 alcohols (Nos 1830 and 1832), 2 aldehydes (Nos 1817 and 1819), 2 acids (Nos 1818 and 1825) and 14 related esters (Nos 1815, 1816, 1820–1824, 1826–1829, 1831, 1833 and 1834). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 32 other members of this group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*). All 32 flavouring agents in that group were concluded to be of no safety concern at the current estimated levels of intake, and the Committee maintained the previously established group ADI of 0–0.5 mg/kg bw, expressed as citral,

for citral (No. 1225), citronellol (No. 1219), geranyl acetate (No. 58), linalool (No. 356) and linalyl acetate (No. 359). Citral and citronellol had already been evaluated by the Committee at its eleventh meeting (Annex 1, reference *14*), at which conditional ADIs[1] of 0–0.25 mg/kg bw and 0–1 mg/kg bw, respectively, were allocated. At the twenty-third meeting of the Committee (Annex 1, reference *50*), citronellol and citral were re-evaluated as part of a group of terpenoid flavouring agents, including geranyl acetate, linalool and linalyl acetate. A group ADI of 0–0.5 mg/kg bw, expressed as citral, was established for citral, geranyl acetate, citronellol, linalool and linalyl acetate on the basis of their clearly defined metabolism, rapid excretion and low toxicity in short-term studies. The Committee maintained, however, that a long-term study was required for at least one member of this group.

At its forty-ninth meeting (Annex 1, reference *131*), the Committee evaluated a group of 26 geranyl, neryl, citronellyl and rhodinyl esters derived from branched-chain terpenoid alcohols and aliphatic acyclic carboxylic acids by the Procedure. Two-year studies of carcinogenicity had been conducted for a mixture of two of these esters, geranyl acetate and citronellyl acetate. The Committee concluded that there were no safety concerns for any of the 26 substances at the low levels of intake arising from their use as flavouring agents and maintained the group ADI for citral, geranyl acetate, citronellol, linalool and linalyl acetate. Likewise, at its fifty-fifth meeting (Annex 1, reference *137*), when the Committee re-evaluated linalool and linalyl acetate as part of a group of 23 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances by the Procedure, the group ADI was maintained. The findings from all previous evaluations were considered in the present evaluation.

Twelve of the 20 flavouring agents in this group have been reported to occur naturally in foods (Nos 1815, 1818, 1820, 1822, 1824–1827, 1830–1832 and 1834). They have been detected in bread, animal fat, a variety of fruits, cinnamon, citrus peel oils, peppermint oil, cheddar cheese, black tea, coffee, white wine, carrot, honey and kelp, for example.

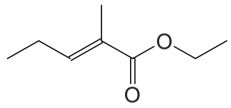
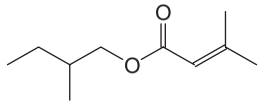
Assessment of dietary exposure

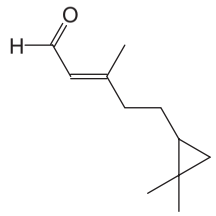
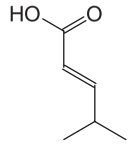
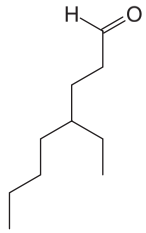
The total annual volume of production of the 20 flavouring agents in this group is approximately 270 kg in Europe, 2200 kg in the USA and 40 kg in Japan. In Europe, the USA as well as Japan, prenyl acetate (No. 1827) makes the biggest contribution to the total annual production volume (67%, 59% and 58%, respectively). The estimated daily per capita intake is the highest for prenyl acetate in the USA (160 µg). For the other flavouring agents, the estimated daily per capita intakes were in the range of 0.01–68 µg. The estimated daily per capita intake of each flavouring agent is reported in Table 6.

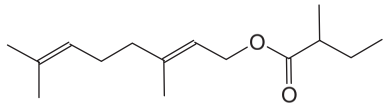
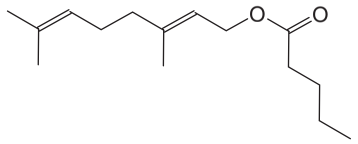
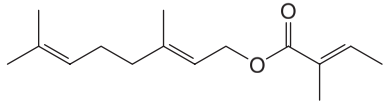
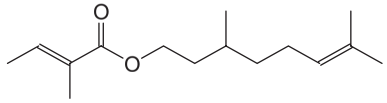
[1] "Conditional ADI", which signifies an ADI with special considerations, is a term no longer used by JECFA.

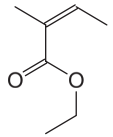
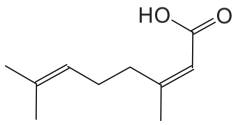
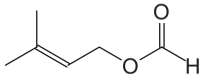
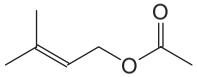
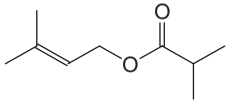
Table 6

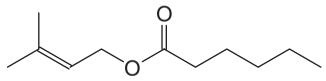
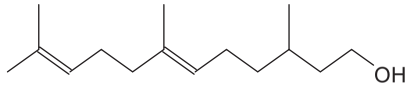
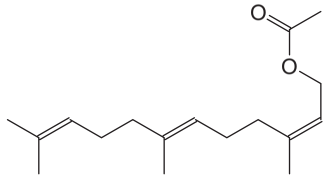
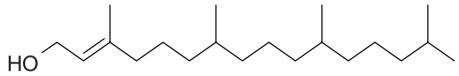
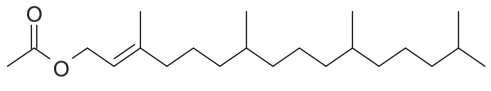
Summary of results of safety evaluations of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters used as flavouring agents[a,b,c]

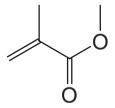
Flavouring agent	No.	CAS No. and structure	*Step A3*[i] Does the estimated intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class I					
Ethyl (*E*)-2-methyl-2-pentenoate	1815	1617-40-9 	No Europe: ND USA: 0.7 Japan: ND	See note 1	No safety concern
2-Methylbutyl 3-methyl-2-butenoate	1816	97890-13-6 	No Europe: ND USA: 24 Japan: ND	See note 1	No safety concern

(±)(*E*,*Z*)-5-(2,2-Dimethylcyclopropyl)-3-methyl-2-pentenal	1817	877-60-1 	No Europe: 0.01 USA: ND Japan: ND	See note 4	No safety concern
(*E*,*Z*)-4-Methylpent-2-enoic acid	1818	10321-71-8 	No Europe: ND USA: 0.05 Japan: ND	See note 2	No safety concern
(±)-4-Ethyloctanal	1819	58475-04-0 	No Europe: 0.01 USA: 0.1 Japan: ND	See note 3	No safety concern

(*E*)-Geranyl 2-methylbutyrate	1820	68705-63-5 	No Europe: 0.01 USA: ND Japan: 0.03	See note 5	No safety concern
(*E*)-Geranyl valerate	1821	10402-47-8 	No Europe: ND USA: 68 Japan: 0.05	See note 5	No safety concern
(*E*)-Geranyl tiglate	1822	7785-33-3 	No Europe: 2 USA: 0.08 Japan: 1	See note 5	No safety concern
(*E*)-Citronellyl 2-methylbut-2-enoate	1823	24717-85-9 	No Europe: 0.02 USA: ND Japan: 0.2	See note 5	No safety concern

(*E*)-Ethyl tiglate	1824	5837-78-5 	No Europe: 5 USA: 0.8 Japan: 1	See note 1	No safety concern
(E,Z)-Geranic acid	1825	459-80-3 	No Europe: 0.04 USA: 0.03 Japan: 0.3	See note 2	No safety concern
Prenyl formate	1826	68480-28-4 	No Europe: 0.01 USA: 0.1 Japan: ND	See note 1	No safety concern
Prenyl acetate	1827	1191-16-8 	No Europe: 19 USA: 160 Japan: 6	See note 1	No safety concern
Prenyl isobutyrate	1828	76649-23-5 	No Europe: 0.01 USA: 0.01 Japan: ND	See note 1	No safety concern

Prenyl caproate	1829	76649-22-4 	No Europe: 0.01 USA: 0.01 Japan: ND	See note 1	No safety concern
(±)-Dihydrofarnesol	1830	51411-24-6 	No Europe: 0.01 USA: 0.5 Japan: ND	See note 4	No safety concern
(E,Z)-3,7, 11-Trimethyldodeca-2,6, 10-trienyl acetate	1831	29548-30-9 	No Europe: 3 USA: 17 Japan: 0.03	See note 5	No safety concern
(E,Z)-Phytol	1832	150-86-7 	No Europe: 0.1 USA: ND Japan: 2	See note 4	No safety concern
(E,Z)-Phytyl acetate	1833	10236-16-5 	No Europe: ND USA: ND Japan: 0.03	See note 5	No safety concern

Structural class II						
Methyl 2-methyl-2-propenoate	1834	80-62-6 	No Europe: 0.03 USA: ND Japan: 0.03	See note 1	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Thirty-two flavouring agents belonging to the same chemical group were previously evaluated by the Committee at its sixty-first meeting (Annex 1, reference *166*).

[b] *Step 1:* Nineteen of the flavouring agents (Nos 1815–1833) in this group were assigned to structural class I, and the remaining flavouring agent (No. 1834) was assigned to structural class II.

[c] *Step 2:* All of the agents in this group are expected to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I and II are 1800 and 540 µg/day, respectively. All intake values are expressed in µg/day.

Notes:

1. Hydrolysed to the corresponding alcohol and carboxylic acid, then participates in the pathway cited in notes 2 and 3.
2. Metabolized primarily via the β-oxidation pathway, yielding shorter-chain carboxylic acids that are subsequently metabolized to carbon dioxide via the tricarboxylic acid pathway.
3. Primarily oxidized to the corresponding carboxylic acid, which may enter the β-oxidation pathway, yielding shorter-chain carboxylic acids that are subsequently metabolized to carbon dioxide via the tricarboxylic acid pathway.
4. Oxidized to corresponding carboxylic acid. The acid may be excreted or undergo ω-oxidation to yield polar polyoxygenated metabolites that are excreted free or conjugated primarily in the urine. If unsaturation is present, the polar polyoxygenated metabolites may also form hydrogenation or hydration metabolites.
5. Hydrolysed to the corresponding alcohol and carboxylic acid, then participates in the pathway cited in notes 2 and 4.

Absorption, distribution, metabolism and elimination

Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters has previously been described in the report of the sixty-first meeting (Annex 1, reference *166*). Additional data on the compounds methyl 2-methyl-2-propenoate (No. 1834) and (*E*,*Z*)-phytol (No. 1832) have now been submitted and are in line with the information described in the report of the sixty-first meeting.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the 20 flavouring agents in this group of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters, the Committee assigned 19 of these flavouring agents (Nos 1815–1833) to structural class I and the remaining flavouring agent (No. 1834) to structural class II.

Step 2. All flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of all 19 flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). According to the Procedure, the safety of these 19 flavouring agents raises no concern when they are used at their current estimated levels of intake.

The estimated daily per capita intake of the flavouring agent in structural class II (i.e. methyl 2-methyl-2-propenoate, No. 1834; synonym methyl methacrylate) is below the threshold of concern (i.e. 540 μg/person per day for class II). However, the Committee noted that there is a structural similarity between this flavouring agent and ethyl methacrylate, a substance reported to be neurotoxic. These two chemicals share a common metabolite, methacrylic acid, which is unlikely to be a neurotoxicant because it is more polar and therefore less likely to cross the blood–brain barrier. Because methyl 2-methyl-2-propenoate was shown to have some neurotoxic properties in rats dosed at 500 mg/kg bw per day by gavage for 21 days, the Committee decided to apply the TTC for structural class III (i.e. 90 μg/person per day), which was derived using data that included neurotoxic compounds. Given that the estimated daily per capita intake of methyl 2-methyl-2-propenoate is even well below this lower threshold of concern, the

Committee concluded that the safety of this flavouring agent raises no concern when it is used at its currently estimated level of intake.

Table 6 summarizes the evaluations of the 20 aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters (Nos 1815–1834) in this group.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters are predicted to be metabolized by hydrolysis and/or oxidative metabolism, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle. These pathways have a high capacity and would not be saturated, even if all flavouring agents were consumed at the same time. Most of the substances in this group that have been evaluated by the Committee at its present meeting and at the sixty-first meeting are predicted or known to be metabolized to common metabolites. Common metabolites are 3-methylcrotonic acid (No. 1204), 2-methylbutyric acid (No. 255), 2-methyl-2-butenoic acid (No. 1205), (*E*,*Z*)-geranic acid (No. 1825), 3,7-dimethyl-6-octenoic acid (No. 1221), phytanic acid, isobutyric acid (No. 253), methacrylic acid, 2-methyl-2-pentenoic acid (No. 1210), (*E*,*Z*)-4-methylpent-2-enoic acid (No. 1818), 4-ethyloctanoic acid (No. 1218) and 3,7,11-trimethyldodeca-2,6,10-trienoic acid. All of these substances are structural class I, except for methacrylic acid, which is structural class II. All calculated combined intakes[1] for each common metabolite in Europe, the USA and Japan for up to five flavouring agents with the highest intakes (i.e. Nos 1200, 1202, 1204, 1816 and 1827 for 3-methylcrotonic acid; Nos 255, 1199, 1816 and 1820 for 2-methylbutyric acid; Nos 1201, 1205, 1822, 1823 and 1824 for 2-methyl-2-butenoic acid; Nos 1223, 1224, 1225, 1821 and 1822 for (*E*,*Z*)-geranic acid; Nos 1219, 1220, 1221 and 1823 for 3,7-dimethyl-6-octenoic acid; Nos 1832 and 1833 for phytanic acid; Nos 253, 1206, 1213 and 1828 for isobutyric acid; Nos 1207 and 1834 for methacrylic acid; Nos 1209, 1210 and 1815 for 2-methyl-2-pentenoic acid; Nos 1208 and 1818 for (*E*,*Z*)-4-methylpent-2-enoic acid; Nos 1218 and 1819 for 4-ethyloctanoic acid; and Nos 1228, 1230 and 1831 for 3,7,11-trimethyldodeca-2,6,10-trienoic acid) were below the threshold of concern (i.e. 1800 and 540 µg/person per day for class I and class II, respectively), except for (*E*,*Z*)-geranic acid.

For (*E*,*Z*)-geranic acid, the estimated combined intakes in the unlikely event that the five flavouring agents with the highest intakes (Nos 1223, 1224, 1225, 1821 and 1822) were to be consumed concurrently on a daily basis were

[1] Combined intake was calculated on a molar basis relative to the formation of the common metabolite.

8585 µg, 8303 µg and 0.75 µg in Europe, the USA and Japan, respectively. However, these five flavouring agents are all expected to be metabolized efficiently. Moreover, approximately 90% of the estimated combined intake for (*E*,*Z*)-geranic acid is accounted for by citral (No. 1225) alone, in both Europe and the USA.

The Committee at its sixty-first meeting concluded that, although high, the estimated intakes for citral in Europe and the USA did not exceed the group ADI of 0–0.5 mg/kg bw, expressed as citral, for citral, geranyl acetate, citronellol, linalool and linalyl acetate, nor did the total estimated combined intakes for all 32 flavouring agents under evaluation (Annex I, reference *166*). The Committee at its present meeting concluded that under the conditions of use as flavouring agents, the combined intakes of the substances leading to a common metabolite would not saturate the metabolic pathways and the combined intakes would not raise safety concerns.

Consideration of secondary components

One member of this group of flavouring agents, (±)(*E*,*Z*)-5-(2,2-dimethylcyclopropyl)-3-methyl-2-pentenal (No. 1817), has an assay value of less than 95%. Information on the safety of the secondary component of this compound is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%). The secondary component in No. 1817, citral (No. 1225), was evaluated by the Committee at its sixty-first meeting (Annex 1, reference *166*) and was considered not to present a safety concern at current estimated levels of intake.

Conclusion

In the previous evaluation of substances in this group, studies of acute toxicity, short-term studies of toxicity (12 days to 28 weeks), long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation.

The Committee concluded that these 20 flavouring agents, which are additions to the group of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters evaluated previously, would not give rise to safety concerns at the current estimated levels of intake.

An addendum to the toxicological monograph was prepared.

4.1.2 *Aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters: additional compounds*

The Committee evaluated a group of flavouring agents consisting of 22 aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters. This group included 1 2-alkenal (No. 1803), 2 2-alken-1-ols (Nos 1793 and 1794), 2 2-alkenoic acids (Nos 1804 and 1805), 14 related alkenoic esters (Nos 1795–1799 and 1806–1814), 2 2-alkenal acetals (Nos 1800 and 1801) and 1 unsaturated methoxy compound (No. 1802) that is predicted to be metabolized to an α,β-unsaturated alcohol. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee previously evaluated 37 other members of this group of flavouring agents at its sixty-third meeting (Annex 1, reference *173*). The findings from these evaluations were considered in the present evaluation. All 37 flavouring agents in that group were concluded to be of no safety concern at the current estimated levels of intake.

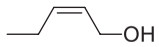
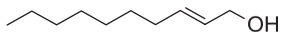
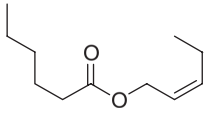
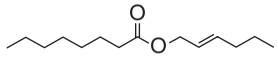
Thirteen of the 22 flavouring agents in this group are natural components of foods (Nos 1793–1795, 1803, 1805–1812 and 1814). They have been primarily detected in a wide variety of fruits, such as apple, strawberry, grape, pear, pineapple, mango, guava, cranberry, plum, passion fruit and papaya, as well as in red and white wine, fruit juices and fruit brandies. They have also been detected to a lesser extent in a number of meats, fish, vegetables and teas.

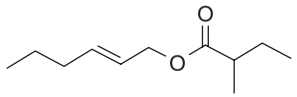
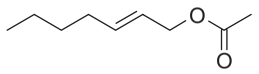
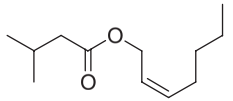
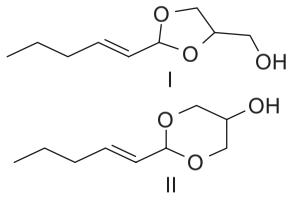
Assessment of dietary exposure

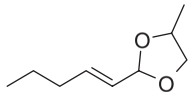
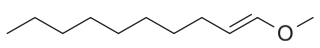
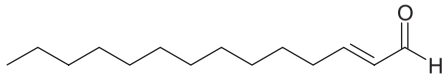
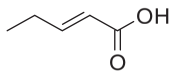
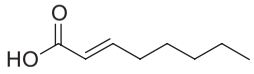
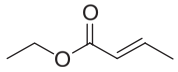
The total annual volume of production of the 22 flavouring agents in this group is approximately 530 kg in Europe, 1500 kg in the USA and 680 kg in Japan. In the USA, approximately 75% of the total annual production volume is accounted for solely by (*E*,*Z*)-methyl 2-nonenoate (No. 1813), whereas (*E*,*Z*)-methyl 2-hexenoate (No. 1809) has the next greatest contribution (approximately 20%). In Europe, more than 90% of the total annual production volume is accounted for by ethyl *trans*-2-hexenoate (No. 1808), ethyl *trans*-2-butenoate (No. 1806), ethyl *trans*-2-octenoate (No. 1812), (*E*,*Z*)-methyl 2-nonenoate (No. 1813) and ethyl *trans*-2-decenoate (No. 1814). In Japan, *trans*-2-hexenal propylene glycol acetal (No. 1801), ethyl *trans*-2-butenoate (No. 1806) and ethyl *trans*-2-decenoate (No. 1814) account for more than 90% of the total annual production volume. The estimated daily per capita intake is the highest for (*E*,*Z*)-methyl 2-nonenoate in the USA (142 μg). For the other flavouring agents, the estimated daily per capita intakes were in the range of 0.01–104 μg. The estimated daily per capita intakes of each agent are reported in Table 7.

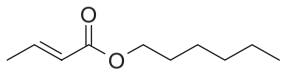
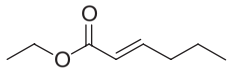
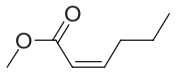
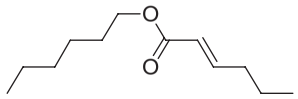
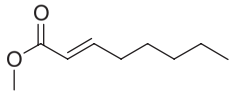
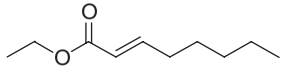
Table 7

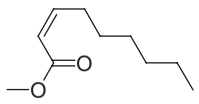
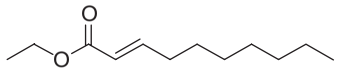
Summary of results of safety evaluations of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does the estimated intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class I					
(Z)-2-Penten-1-ol	1793	20273-24-9 	No Europe: 0.6 USA: ND Japan: ND	See note 1	No safety concern
(E)-2-Decen-1-ol	1794	18409-18-2 	No Europe: 0.01 USA: ND Japan: 0.03	See note 1	No safety concern
(*Z*)-Pent-2-enyl hexanoate	1795	74298-89-8 	No Europe: 0.07 USA: ND Japan: ND	See note 2	No safety concern
(*E*)-2-Hexenyl octanoate	1796	85554-72-9 	No Europe: 0.01 USA: ND Japan: 0.4	See note 2	No safety concern

trans-2-Hexenyl 2-methylbutyrate	1797	94089-01-7 	No Europe: ND USA: ND Japan: 0.2	See note 2	No safety concern
Hept-*trans*-2-en-1-yl acetate	1798	16939-73-4 	No Europe: 0.01 USA: 0.01 Japan: ND	See note 2	No safety concern
(*E*,*Z*)-Hept-2-en-1-yl isovalerate	1799	253596-70-2 	No Europe: 0.01 USA: 5 Japan: ND	See note 2	No safety concern
trans-2-Hexenal glyceryl acetal	1800	214220-85-6 (E,I) 897630-96-5 (Z,I) 897672-50-3 (E,II) 897672-51-4 (Z,II) 	No Europe: ND USA: ND Japan: 3	See note 3	No safety concern

trans-2-Hexenal propylene glycol acetal	1801	94089-21-1 	No Europe: ND USA: ND Japan: 104	See note 3	No safety concern
cis- and trans-1-Methoxy-1-decene	1802	79930-37-3 	No Europe: 0.01 USA: 0.1 Japan: ND	See note 4	No safety concern
(*E*)-Tetradec-2-enal	1803	51534-36-2 	No Europe: 0.01 USA: 0.07 Japan: ND	See note 5	No safety concern
(*E*)-2-Pentenoic acid	1804	13991-37-2 	No Europe: 0.01 USA: ND Japan: 0.03	See note 6	No safety concern
(E)-2-Octenoic acid	1805	1871-67-6 	No Europe: 0.01 USA: ND Japan: 0.03	See note 6	No safety concern
Ethyl *trans*-2-butenoate	1806	10544-63-5 	No Europe: 12 USA: 5 Japan: 35	See note 2	No safety concern

Hexyl 2-butenoate	1807	19089-92-0 	No Europe: 2 USA: ND Japan: 0.03	See note 2	No safety concern
Ethyl *trans*-2-hexenoate	1808	27829-72-7 	No Europe: 18 USA: 0.02 Japan: 10	See note 2	No safety concern
(*E*,*Z*)-Methyl 2-hexenoate	1809	2396-77-2 	No Europe: 0.03 USA: 35 Japan: 0.08	See note 2	No safety concern
Hexyl *trans*-2-hexenoate	1810	33855-57-1 	No Europe: 0.01 USA: 0.2 Japan: ND	See note 2	No safety concern
Methyl *trans*-2-octenoate	1811	7367-81-9 	No Europe: 0.2 USA: 0.3 Japan: 0.03	See note 2	No safety concern
Ethyl *trans*-2-octenoate	1812	7367-82-0 	No Europe: 10 USA: 0.08 Japan: 0.03	See note 2	No safety concern

(*E*,*Z*)-Methyl 2-nonenoate	1813	111-79-5 	No Europe: 7 USA: 142 Japan: 1	See note 2	No safety concern
Ethyl *trans*-2-decenoate	1814	7367-88-6 	No Europe: 6 USA: 0.02 Japan: 26	See note 2	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Thirty-seven flavouring agents belonging to the same chemical group were previously evaluated by the Committee at its sixty-third meeting (Annex 1, reference *173*).

[b] *Step 1:* All 22 flavouring agents in this group are in structural class I.

[c] *Step 2:* All of the agents in this group are expected to be metabolized to innocuous products.

[d] The threshold for human intake for structural class I is 1800 µg/person per day. All intake values are expressed in µg/day.

Notes:

[1] Oxidized to aldehydes and acids, which metabolize completely in the fatty acid β-oxidation pathway.

[2] Hydrolysed to corresponding alcohols and acids, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle.

[3] Hydrolysed to corresponding aldehydes and alcohols, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle.

[4] *O*-Demethylated, followed by oxidation to aldehyde and acid and complete metabolism in the fatty acid β-oxidation pathway or the tricarboxylic acid cycle.

[5] Oxidized to acids, which may undergo β-oxidative cleavage and complete metabolism via the tricarboxylic acid cycle. Alternatively, may undergo glutathione conjugation and excretion as mercapturic acid derivatives.

[6] Undergoes β-oxidative cleavage and complete metabolism via the tricarboxylic acid cycle.

Absorption, distribution, metabolism and elimination

Information on the hydrolysis, absorption, distribution, metabolism and elimination of flavouring agents belonging to the group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters has previously been described in the report of the sixty-third meeting (Annex 1, reference *173*). No relevant additional data have been reported since that meeting.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the 22 flavouring agents in this group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters, the Committee assigned all 22 (Nos 1793–1814) to structural class I.

Step 2. All flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of all 22 flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). According to the Procedure, the safety of these 22 flavouring agents raises no concern when they are used at their current estimated levels of intake.

Table 7 summarizes the evaluations of the 22 aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters (Nos 1793–1814) in this group.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters are predicted to be metabolized by hydrolysis and/or oxidative metabolism, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle. These pathways have a high capacity and would not be saturated, even if all flavouring agents were consumed at the same time. Most of the substances in this group that have been evaluated by the Committee at its present meeting and at the sixty-third meeting are predicted or known to be metabolized to common metabolites. Common metabolites (and their precursors) are 2-butenoic acid (No. 1371), 2-pentenoic acid (No. 1804), 2-hexenoic acid (No. 1361), 2-heptenoic acid (No. 1373), 2-octenoic acid (No. 1805), 2-nonenoic acid (No. 1380) and 2-decenoic acid (No. 1372), all of which are structural class I. When calculating for each common metabolite the

combined intakes[1] in Europe, the USA and Japan for up to five flavouring agents with the highest intakes (i.e. Nos 1371, 1806 and 1807 for 2-butenoic acid; Nos 1364, 1793, 1795 and 1804 for 2-pentenoic acid; Nos 1353, 1354, 1355, 1361 and 1801 for 2-hexenoic acid; Nos 1360, 1373, 1798 and 1799 for 2-heptenoic acid; Nos 1363, 1367, 1370, 1811 and 1812 for 2-octenoic acid; Nos 1362, 1365, 1369, 1380 and 1813 for 2-nonenoic acid; and Nos 1348, 1349, 1372, 1794 and 1814 for 2-decenoic acid), they were all below the threshold of concern (i.e. 1800 μg/person per day for class I). An additional consideration is that these common metabolites are part of a homologous series of 2-alkenoic acids; the combined intakes of the five flavouring agents in this homologous series with the highest intakes in Europe, the USA and Japan (i.e. Nos 1353, 1354, 1355, 1801 and 1813) would not exceed the human intake threshold of concern (i.e. 1800 μg/person per day for class I). The Committee concluded that under the conditions of use as flavouring agents, the combined intakes of the substances leading to a common metabolite would not saturate the metabolic pathways and the combined intakes would not raise safety concerns.

Consideration of secondary components

Three members of this group of flavouring agents, *trans*-2-hexenal glyceryl acetal (No. 1800), hexyl *trans*-2-hexenoate (No. 1810) and methyl *trans*-2-octenoate (No. 1811), have assay values of less than 95%. Information on the safety of the secondary components of these three compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%). The secondary components of *trans*-2-hexenal glyceryl acetal, 3-hexenal glyceryl acetal and hexanal glyceryl acetal, are expected to share the same metabolic fate. The secondary component of hexyl *trans*-2-hexenoate, hexyl *trans*-3-hexenoate, is expected to share the same metabolic fate as the primary substance, as is the secondary component of methyl *trans*-2-octenoate, methyl *trans*-3-octenoate. None of the secondary components is considered to present a safety concern at current estimated levels of intake of the flavouring agents.

Conclusion

In the previous evaluation of substances in this group, studies of acute toxicity, short-term studies of toxicity (2–13 weeks), long-term studies of toxicity and carcinogenicity and studies of genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation.

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[1] Combined intake was calculated on a molar basis relative to the formation of the common metabolite.

The Committee concluded that these 22 flavouring agents, which are additions to the group of aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters evaluated previously, would not give rise to safety concerns at the current estimated levels of intake.

An addendum to the toxicological monograph was prepared.

4.1.3 *Aliphatic secondary alcohols, ketones and related esters: additional compounds*

The Committee evaluated a group of 17 aliphatic secondary alcohols, ketones and related esters, including 3 secondary alcohols (Nos 1841, 1842 and 1850), 8 ketones (Nos 1839, 1840, 1843–1845, 1848, 1849 and 1851) and 6 esters of secondary alcohols (Nos 1835–1838, 1846 and 1847). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents had been evaluated previously by the Committee.

The Committee evaluated 39 other members of this group of flavouring agents at its fifty-ninth meeting (Annex 1, reference *160*). All 39 substances in that group were concluded to be of no safety concern based on current estimated levels of intake.

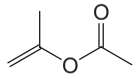
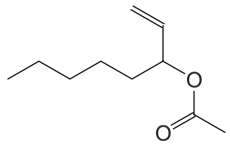
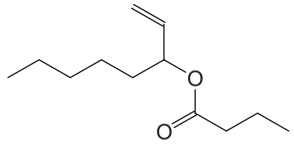
Eleven of the 17 flavouring agents (Nos 1835–1837, 1842–1846, 1848, 1849 and 1851) in this group are natural components of foods. They have been detected in spearmint oil, mentha oils, origanum, *Capsicum annuum*, anise hyssop, mushroom, tomato, celery leaves and stalks, potato, lemon peel oil, melon, banana, guava, chicken, cured pork, beer, rum, tea, and Gruyere and parmesan cheeses (*17*).

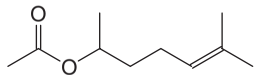
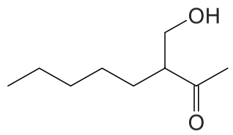
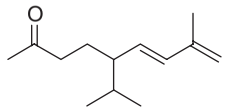
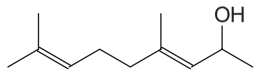
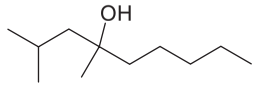
Assessment of dietary exposure

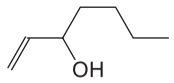
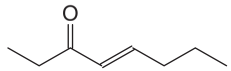
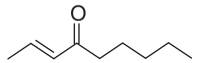
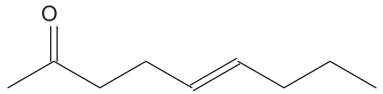
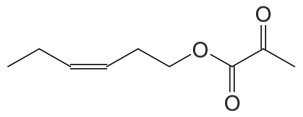
The total annual production volume of the 17 aliphatic unsaturated alcohols, ketones and related esters is approximately 42 kg in Europe, 421 kg in the USA and 192 kg in Japan (*14*, *15*, *16*). Approximately 95% of the total annual volume of production in Europe is accounted for by 1-octen-3-yl acetate (No. 1836) and 3-(hydroxymethyl)-2-octanone (No. 1839); approximately 88% in the USA is accounted for by isopropenyl acetate (No. 1835) and 1-octen-3-yl acetate (No. 1836); and approximately 75% in Japan is accounted for by (*Z*)-3-hexenyl 2-oxopropionate (No. 1846). The estimated daily per capita intake of each flavouring agent is reported in Table 8. Annual volumes of production of this group of flavouring agents are summarized in Table 9.

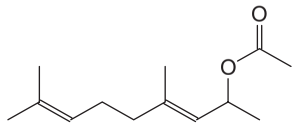
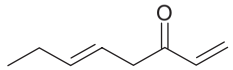
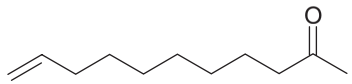
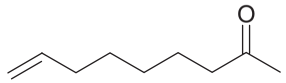
Table 8

Summary of results of safety evaluations of aliphatic secondary alcohols, ketones and related esters used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class I					
Isopropenyl acetate	1835	108-22-5 	No Europe: 0.01 USA: 19 Japan: ND	See notes 1 and 2	No safety concern
1-Octen-3-yl acetate	1836	2442-10-6 	No Europe: 2 USA: 28 Japan: 0.2	See notes 1 and 2	No safety concern
1-Octen-3-yl butyrate	1837	16491-54-6 	No Europe: 0.01 USA: 0.06 Japan: 0.03	See notes 1 and 2	No safety concern

6-Methyl-5-hepten-2-yl acetate	1838	19162-00-6 	No Europe: 0.01 USA: 0.1 Japan: ND	See notes 1, 2 and 4	No safety concern
3-(Hydroxymethyl)-2-octanone	1839	59191-78-5 	No Europe: 3 USA: 0.6 Japan: ND	See note 1	No safety concern
(±)-[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one	1840	2278-53-7 	No Europe: 0.01 USA: 1 Japan: ND	See notes 1, 3 and 4	No safety concern
(±)-*cis*- and *trans*-4,8-Dimethyl-3,7-nonadien-2-ol	1841	67845-50-5 	No Europe: 0.01 USA: 0.1 Japan: ND	See note 1	No safety concern
2,4-Dimethyl-4-nonanol	1850	74356-31-3 	No Europe: ND USA: ND Japan: 12.4	See note 1	No safety concern

Structural class II					
(±)-1-Hepten-3-ol	1842	4938-52-7 	No Europe: 0.01 USA: 1 Japan: 0.03	See note 1	No safety concern
(E,Z)-4-Octen-3-one	1843	14129-48-7 	No Europe: ND USA: 0.6 Japan: ND	See notes 1 and 3	No safety concern
(*E*)-2-Nonen-4-one	1844	2773-70-0 	No Europe: ND USA: 1 Japan: ND	See notes 1 and 3	No safety concern
(*E*)-5-Nonen-2-one	1845	27039-84-5 	No Europe: ND USA: 0.4 Japan: ND	See notes 1 and 3	No safety concern
(*Z*)-3-Hexenyl 2-oxopropionate	1846	68133-76-6 	No Europe: 0.1 USA: 0.01 Japan: 38	See notes 1 and 2	No safety concern

(±)-*cis*- and *trans*-4,8-Dimethyl-3,7-nonadien-2-yl acetate	1847	91418-25-6 	No Europe: 0.01 USA: 0.2 Japan: ND	See notes 1 and 2	No safety concern
(E)-1,5-Octadien-3-one	1848	65213-86-7 	No Europe: ND USA: ND Japan: 0.03	See note 1	No safety concern
10-Undecen-2-one	1849	36219-73-5 	No Europe: ND USA: ND Japan: 0.3	See note 1	No safety concern
8-Nonen-2-one	1851	5009-32-5 	No Europe: ND USA: ND Japan: 0.05	See note 1	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Thirty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *160*).

[b] *Step 1*: Eight of the flavouring agents (Nos 1835-1841 and 1850) are in structural class I, and nine flavouring agents (Nos 1842–1849 and 1851) are in structural class II.

[c] *Step 2*: All the agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I and II are 1800 and 540 µg/day, respectively. All intake values are expressed in µg/day. The combined per capita intakes of the flavouring agents in a homologous series of unsaturated secondary alcohols or with the common metabolite 1-octen-3-ol are 296, 47 and 0.5 µg/person per day in Europe, the USA and Japan, respectively. The combined per capita intakes of the flavouring agents in a homologous series of branched-chain unsaturated secondary alcohols or with the common metabolite 6-methyl-5-hepten-ol are 119 and 45 µg/person per day in Europe and the

USA, respectively. The combined per capita intakes of the flavouring agents in a homologous series of branched-chain diene secondary alcohols or with the common metabolite (±)-*trans*- and *cis*-4,8-dimethyl-3,8-nonadien-2-ol are 7 and 8 µg/person per day in Europe and the USA, respectively. The combined per capita intakes of the flavouring agents in a homologous series of linear unsaturated secondary alcohols are 15.3 and 1.6 µg/person per day in Europe and the USA, respectively. The combined per capita intakes of the flavouring agents in a homologous series of unsaturated secondary alcohols are 0.01 and 1 µg/person per day in Europe and the USA, respectively. The combined per capita intake of the flavouring agents in a homologous series of linear unsaturated non-conjugated secondary alcohols is 0.4 µg/person per day in Japan.

Notes:

1. Detoxified by reduction of the ketone followed by glucuronic acid conjugation of the corresponding alcohol or direct glucuronic acid conjugation of the secondary alcohol.
2. Detoxified by hydrolysis of ester and glucuronic acid conjugation of the resulting alicyclic alcohol and complete oxidation of the carboxylic acid.
3. Detoxified by reduction of the ketone functional group followed by glucuronic acid conjugation of the resulting alcohol and glutathione conjugation of the parent ketone.
4. Detoxified by reduction of the ketone and alkyl side-chain oxidation and excretion.

Table 9

Annual volumes of production of aliphatic secondary alcohols, ketones and related esters used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Intake[b]		Annual volume of natural occurrence in foods (kg)[c]	Consumption ratio[d]
		µg/day	µg/kg bw per day		
Isopropenyl acetate (1835)					
Europe	0.1	0.01	0.0002		
USA	152	19	0.3	+	NA
Japan	ND	ND	ND		
1-Octen-3-yl acetate (1836)					
Europe	16	2	0.03		
USA	227	28	0.5	370	1.6
Japan	0.6	0.2	0.003		
1-Octen-3-yl butyrate (1837)					
Europe	0.1	0.01	0.0002		
USA	0.5	0.06	0.001	+	NA
Japan	0.1	0.03	0.0004		
6-Methyl-5-hepten-2-yl acetate (1838)					
Europe	0.1	0.01	0.0002		
USA	1	0.1	0.002	–	NA
Japan	ND	ND	ND		
3-(Hydroxymethyl)-2-octanone (1839)					
Europe	24	3	0.04		
USA	5	0.6	0.009	–	NA
Japan	ND	ND	ND		
(±)-[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one (1840)					
Europe	0.1	0.01	0.0002		
USA	5	1	0.01	–	NA
Japan	ND	ND	ND		
(±)-*cis*- and *trans*-4,8-Dimethyl-3,7-nonadien-2-ol (1841)					
Europe	0.1	0.01	0.0002		
USA	1	0.1	0.002	–	NA
Japan	ND	ND	ND		
(±)-1-Hepten-3-ol (1842)					
Europe	0.1	0.01	0.0002		
USA	10	1	0.02	1345	135
Japan	0.1	0.03	0.0004		
(E,Z)-4-Octen-3-one (1843)					
Europe	ND	ND	ND		
USA	5	0.6	0.01	+	NA
Japan	ND	ND	ND		
(*E*)-2-Nonen-4-one (1844)					

Europe	ND	ND	ND		
USA	10	1	0.02	9.3	0.9
Japan	ND	ND	ND		
(*E*)-5-Nonen-2-one (1845)					
Europe	ND	ND	ND		
USA	3	0.4	0.01	+	NA
Japan	ND	ND	ND		
(*Z*)-3-Hexenyl 2-oxopropionate (1846)					
Europe	1.2	0.1	0.002		
USA	0.1	0.01	0.00020	+	NA
Japan	143	38	0.6		
(±)-*cis*- and *trans*-4,8-Dimethyl-3,7-nonadien-2-yl acetate (1847)					
Europe	0.1	0.01	0.0002		
USA	2	0.2	0.004	–	NA
Japan	ND	ND	ND		
(E)-1,5-Octadien-3-one (1848)					
Europe	ND	ND	ND		
USA	ND	ND	ND	+	NA
Japan	0.10	0.03	0.0004		
10-Undecen-2-one (1849)					
Europe	ND	ND	ND		
USA	ND	ND	ND	+	NA
Japan	1	0.3	0.004		
2,4-Dimethyl-4-nonanol (1850)					
Europe	ND	ND	ND		
USA	ND	ND	ND	–	NA
Japan	47	12.4	0.2		
8-Nonen-2-one (1851)					
Europe	ND	ND	ND		
USA	ND	ND	ND	+	NA
Japan	0.2	0.05	0.001		
Total					
Europe	42				
USA	421				
Japan	192				

NA, not available; ND, no intake data reported; +, reported to occur naturally in foods (*17*), but no quantitative data; –, not reported to occur naturally in foods.

[a] From references *14*, *15* and *16*. Total poundage values of <0.1 kg reported in the surveys (*14*, *15*, *16*) have been truncated to one place following the decimal point (0.1 kg).

[b] Intake (µg/person per day) calculated as follows: [(annual volume, kg) × (1 × 109 µg/kg)]/[population × survey correction factor × 365 days], where population (10%, "consumers only") = 32 × 106 for Europe, 28 × 106 for the USA and 13 × 106 for Japan; where survey correction factor = 0.8 for the surveys by the USA, Europe and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*14*, *15*, *16*).

Intake (µg/kg bw per day) calculated as follows: (µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] Quantitative data for the USA reported by Stofberg & Grundschober (*18*).

[d] The consumption ratio is calculated as follows: (annual consumption via food, kg)/(most recent reported volume as a flavouring substance, kg).

Absorption, distribution, metabolism and elimination

The aliphatic esters in this group are hydrolysed to the corresponding secondary alcohols. Secondary alcohols and their corresponding ketones are interconvertible under physiological conditions. In the principal excretion pathway, the ketones are reduced to the corresponding secondary alcohols, which are subsequently conjugated with glucuronic acid and excreted mainly in the urine (Annex 1, references *138* and *160*).

If the ketone carbonyl function is located at the 2-position (i.e. a methyl ketone), the methyl group may undergo α-hydroxylation and subsequent oxidation to eventually yield a corresponding ketocarboxylic acid. The ketoacids are intermediary metabolites (e.g. α-ketoacids) that may undergo oxidative decarboxylation to yield carbon dioxide and simple aliphatic carboxylic acids. The acid may be metabolized in the fatty acid pathway and citric acid cycle (Annex 1, reference *138*). If the substance is an α,β-unsaturated ketone or secondary alcohol that is oxidized to an α,β-unsaturated ketone, it may be conjugated with glutathione and excreted in urine as the mercapturic acid derivative.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned eight flavouring agents (Nos 1835–1841 and 1850) to structural class I (*2*). The remaining nine flavouring agents (Nos 1842–1849 and 1851) were assigned to structural class II (*2*).

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all of the flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of the eight flavouring agents in structural class I are below the threshold of concern (i.e. 1800 µg/person per day for class I). The estimated daily per capita intakes of the nine flavouring agents in structural class II are below the threshold of concern (i.e. 540 µg/person per day for class II). According to the Procedure, the safety of these 17 flavouring agents raises no concern when they are used at their current estimated levels of intake.

Table 8 summarizes the evaluations of the 17 aliphatic secondary alcohols, ketones and related esters (Nos 1835–1851) in this group.

Toxicological data

Studies of acute toxicity report median lethal dose (LD_{50}) values of 850 and 3500 mg/kg bw for 1-octen-3-yl acetate (No. 1836) in rats and 1-octen-3-yl butyrate (No. 1837) in mice, respectively (*19*, *20*, *21*). These data and those previously evaluated by the Committee at its fifty-ninth meeting demonstrate the low acute oral toxicity of these 17 flavouring agents (Annex 1, reference *160*).

Consideration of combined intakes from use as flavouring agents[1]

In the unlikely event that the flavouring agents in a homologous series of unsaturated secondary alcohols or with a common metabolite of 1-octen-3-ol, in structural class II, of which the highest intakes correspond to Nos 1148, 1150–1152, 1836, 1837 and 1842 in Europe, the USA and Japan, were to be consumed concurrently on a daily basis, the estimated combined intakes of 296, 47 and 0.5 μg/person per day in Europe, the USA and Japan, respectively, would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

In the unlikely event that the flavouring agents in a homologous series of branched-chain unsaturated secondary alcohols or with a common metabolite of 6-methyl-5-hepten-2-ol, in structural class II, of which the highest intakes correspond to Nos 1119, 1120 and 1838 in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes of 119 and 45 μg/person per day in Europe and the USA, respectively, would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

In the unlikely event that the flavouring agents in a homologous series of branched-chain diene secondary alcohols or with a common metabolite of (±)-*cis*- and *trans*-4,8-dimethyl-3,8-nonadien-2-ol, in structural class II, of which the highest intakes correspond to Nos 1137, 1841 and 1847 in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes of 7 and 8 μg/person per day in Europe and the USA, respectively, would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

In the unlikely event that the flavouring agents in a homologous series of linear unsaturated secondary alcohols, in structural class II, of which the highest intakes correspond to Nos 1125 and 1843 in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes of 15.3 and 1.6 μg/person per day in Europe and the USA,

[1] Combined intake was calculated on a molar basis relative to the formation of a common metabolite.

respectively, would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

In the unlikely event that the flavouring agents in a homologous series of unsaturated secondary alcohols, in structural class II, of which the highest intakes correspond to Nos 1126 and 1844 in Europe and the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes of 0.01 and 1 μg/person per day in Europe and the USA, respectively, would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

In the unlikely event that the flavouring agents in a homologous series of linear unsaturated non-conjugated secondary alcohols, in structural class II, of which the highest intakes correspond to Nos 1849 and 1851 in Japan, were to be consumed concurrently on a daily basis, the estimated combined intake of 0.4 μg/person per day in Japan would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

Consideration of secondary components

Two members of this group of flavouring agents, 3-(hydroxymethyl)-2-octanone (No. 1839) and 2,4-dimethyl-4-nonanol (No. 1850), have an assay value of less than 95%. Information on the safety of the secondary components of these compounds is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%). The secondary component in No. 1839 (3-methylene-2-octanone, No. 1149) had been evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *160*) and was concluded to be of no safety concern at current estimated levels of intake as a flavouring agent. The secondary components of No. 1850 (2,6,8-trimethyl-6-hydroxy-4-nonanone, *cis*-2,6,8-trimethyl- 5-nonen-4-one and *trans*-2,6,8-trimethyl-5-nonen-4-one) are expected to share the same metabolic fate as the primary substance. None of these secondary components is considered to present a safety concern at current estimated levels of intake of the flavouring agents.

Conclusion

In the previous evaluation of substances in this group, studies of acute toxicity, short-term studies of toxicity and studies of genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation (Annex 1, reference *160*).

The Committee concluded that the addition of these 17 flavouring agents to the group of alcohols, ketones and related esters evaluated previously does not raise any safety concerns at the current estimated levels of intake.

No addendum to the toxicological monograph was prepared.

4.1.4 *Alkoxy-substituted allylbenzenes present in foods and essential oils and used as flavouring agents*

The common structural features of this group of six substances are an allylbenzene nucleus and one or more alkoxy ring substituents. All have an alkoxy substitution at the *para* position. Three members of the group contain a 3,4-methylenedioxy substituent and may have additional methoxy substituents: safrole (3,4-methylenedioxyallylbenzene, No. 1792), myristicin (5-methoxy-3,4-methylenedioxyallylbenzene, No. 1791) and apiole (2,5-dimethoxy-3,4-methylenedioxyallylbenzene, No. 1787). Three other substances contain only methoxy substituents: estragole (4-methoxyallylbenzene, No. 1789), methyl eugenol (3,4-dimethoxyallylbenzene, No. 1790) and elemicin (3,4,5-trimethoxyallylbenzene, No. 1788). Because of the widespread occurrence of alkoxy-substituted allylbenzenes in food, mainly in spices and herbs but also in certain vegetables and fruits, these substances are referred to by their common names.

Estragole was reviewed by the Committee at its twenty-third and twenty-fifth meetings (Annex 1, references *50* and *56*), and safrole was reviewed at the fifth and twenty-fifth meetings (Annex 1, references *5* and *56*). For estragole, no ADI was allocated at the twenty-fifth meeting, and the Committee requested additional long-term studies for evaluation of carcinogenic potential. For safrole, the Committee concluded at its twenty-fifth meeting that flavouring agents containing safrole or isosafrole as the principal flavour-active ingredient should not be used as food additives; and that it is not practicable to advocate the discontinuance of spices containing safrole or isosafrole as minor constituents (e.g. nutmeg, mace and cinnamon). However, the Committee at that meeting concluded that when these spices were used, the amounts of safrole and isosafrole in the finished product should be kept as low as practicable.

Many of these substances are genotoxic and carcinogenic in animals. In accordance with general principles elaborated by the Committee at its forty-ninth meeting (Annex 1, reference *131*), the Committee at its present meeting concluded that these substances could not be evaluated using the Procedure.

Assessment of dietary exposure

Dietary exposure to these six substances in Europe, Asia and the USA occurs mainly by consumption of foods, principally spices and herbs, in which they occur and by consumption of essential oils that are isolated from these foods. Exposures to myristicin and safrole occur mainly by consumption of nutmeg, mace, parsley, parsley seed oil and star anise. Exposure to apiole is predominantly from consumption of the herb parsley. Exposures to the three methoxy-substituted allylbenzenes (estragole, methyl eugenol and elemicin)

also occur principally from consumption of spices and spice oils. Exposure to estragole occurs primarily from consumption of foods containing sweet basil, fennel and anise or their essential oils; exposure to methyl eugenol is from nutmeg, allspice, sweet basil and fennel; and exposure to elemicin is from nutmeg, mace, tarragon and parsley seed oil.

The range of exposure to alkoxy-substituted allylbenzenes from spices and spice oils is generally similar, with the mean exposures to safrole, myristicin, estragole and methyl eugenol in the range of 63–166 μg/person per day. Based on the highest reported levels of spice oil in the spice and the highest reported concentration of the alkoxy-substituted allylbenzenes in the oil, the maximum dietary exposure levels for the same four substances are in the range of 424–569 μg/person per day in the USA. Based primarily on EU import data for nutmeg and mace, the predicted maximum dietary exposures to safrole and myristicin are 879 and 684 μg/person per day, respectively. The predicted maximum dietary exposures to the remaining two alkoxy-substituted allylbenzenes (elemicin and apiole) from all sources make a minor contribution to overall exposure. On the basis of typical patterns of consumption, the average daily exposure to each of these substances from spices, foods and essential oils and as intentionally added flavour ingredients does not exceed 1 mg/day (17 μg/kg bw per day). For the four alkoxy-substituted allylbenzenes with the highest production volume, exposures from spice sources normally exceed exposures from spice oil sources by at least a factor of 10.

Only estragole and methyl eugenol are used as flavouring agents, and use is limited to the USA. Based on annual production volumes of 491 kg/year for estragole and 77 kg/year for methyl eugenol, per capita intakes for the whole population as flavouring agents for the USA are 5 and 0.8 μg/day, respectively.

These six alkoxy-substituted allylbenzenes have been and will continue to be consumed as a normal part of a traditional diet. They occur in highest concentrations in spices, which are generally consumed at low levels in food. Recent data indicating that methyl eugenol is essentially ubiquitous in samples of human serum establish the fact that humans are regularly exposed to this substance in the diet.

Evaluation of toxicological data

Most of the data in rodents indicate that at relatively high doses, several alkoxy-substituted allylbenzenes exhibit hepatocarcinogenic potential and DNA binding in the liver. In addition, neuroendocrine tumours of the stomach were induced by estragole and methyl eugenol. Current scientific evidence supports a non-linear relationship between dose and the potential for

carcinogenicity of these substances. The mechanism or mechanisms by which these substances induce cancer in animals have not been established.

The current database for rodents has a number of limitations that have an impact on its direct application to human risk assessment, including those listed below.

Interpretation of carcinogenicity data from studies in which high doses were administered by gavage. Many of the studies of carcinogenicity after oral administration involved gavage. Gavage administration of high doses delivered as a bolus coupled with rapid absorption represents an acute high-level exposure of the liver, the main target organ. For many other substances, it has been shown that dosing by gavage can produce metabolic and toxicological effects that do not occur when the same daily dose is given in the diet.

Nature of the dose–response relationship for hepatocarcinogenicity. Hepatocarcinogenicity in rodents has been reported only at high doses, usually in excess of the maximum tolerable dose (MTD).

At high doses, there is a dose-dependent saturation of the principal pathways of metabolic detoxication, leading to an increased proportion of the dose undergoing metabolic activation of the allyl side-chain to the sulfate conjugates of 1′-hydroxy metabolites, which are the putative carcinogenic products. In addition, there is evidence for auto-induction of cytochrome P450 (CYP)-mediated metabolic activation at high doses.

DNA adducts have been quantified in rodents and, in some studies, appear to occur with a linear dose–response relationship over a wide range of doses, but the relationship of DNA adducts to hepatocarcinogenesis has not been studied in detail. Information is lacking on the efficiency of repair of these adducts and on the dose–response relationship for DNA repair by either rodent or human hepatocytes. Studies that further investigate the relationship between DNA adduct formation and toxicity, especially bioindicators of carcinogenicity, would provide valuable information.

Doses producing hepatotoxicity have the potential to enhance carcinogenicity by induced liver cell regeneration, which serves to fix DNA damage as mutations. Studies are needed to investigate bioindicators of neoplasia in rodents, at doses below and including those that produce hepatotoxicity.

Non-relevance of neuroendocrine gastric tumours to humans. Rodents have high basal levels of blood gastrin and a high density of neuroendocrine cells in the stomach glandular mucosa. With parietal cell injury and reduced production of hydrochloric acid, gastrin levels rise markedly and stimulate proliferation of responsive neuroendocrine cells. A variety of antisecretory

drugs produce neuroendocrine cell neoplasms in rodents, but similar responses do not occur in humans.

Relevance of the toxicity data to the ingestion of spices. Data are needed to clarify whether the dose–response data in rodents for single compounds are relevant to their presence in natural spices. Recent in vitro data suggest that other components of natural spices might modulate the bioactivation and/or detoxication of these substances, such that the toxicity data relate to the use of these substances as flavouring agents but not to their presence in natural spices.

Epidemiological studies on spice ingestion. Spices containing these substances have been ingested by humans for millennia, without apparent harm. However, structured epidemiological research on the possibility of an association between spice consumption and hepatic cancer in humans is lacking.

Conclusion

The Committee concluded that the data reviewed on the six alkoxy-substituted allylbenzenes provide evidence of toxicity and carcinogenicity to rodents given high doses for several of these substances. A mechanistic understanding of these effects and their implications for human risk have yet to be fully explored and will have a significant impact on the assessment of health risks from alkoxy-substituted allylbenzenes at the concentrations at which they occur in food. Further research is needed to assess the potential risk to human health from low-level dietary exposure to alkoxy-substituted allylbenzenes present in foods and essential oils and used as flavouring agents.

A toxicological monograph was prepared.

4.1.5 *Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids: additional compounds*

The Committee evaluated a group of seven flavouring agents, all of which were esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents established by the Committee at its forty-ninth meeting (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated.

The Committee evaluated 66 other members of this group of flavouring agents at its forty-ninth meeting (Annex 1, reference *132*). All 66 substances in that group were concluded to be of no safety concern based on current estimated levels of intake.

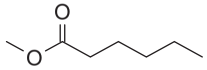
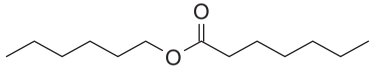
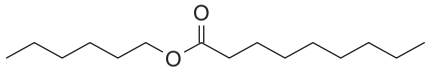
Three of the seven flavouring agents in this group are natural components of foods (Nos 1871, 1872 and 1874). They have been detected in apples, bananas, apricots, pineapples, strawberries, potatoes, tomatoes, mushrooms, a wide variety of cheeses, butter, milk, beef, mutton, wine, brandy, coffee, tea, honey and oysters (*17*).

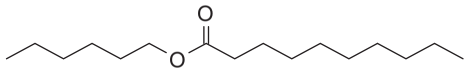
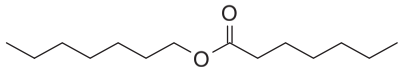
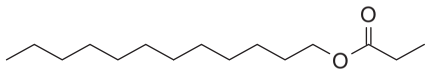
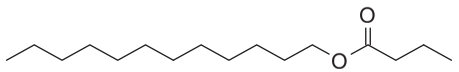
Assessment of dietary exposure

The total annual volume of production of this group of esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids is approximately 2334 kg in Europe, 336 kg in the USA and 801 kg in Japan (*14*, *15*, *16*). More than 80% of the annual production volume in Japan and all of the production volume in the USA are accounted for by methyl hexanoate (No. 1871). The estimated daily per capita intake of each flavouring agent is reported in Table 10. Annual volumes of production of this group of flavouring agents are summarized in Table 11.

Table 10

Summary of results of safety evaluations of esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class I					
Methyl hexanoate	1871	106-70-7 	No Europe: 235 USA: 41 Japan:182	See note 1	No safety concern
Hexyl heptanoate	1872	1119-06-8 	No Europe: ND USA: ND Japan: 0.04	See note 1	No safety concern
Hexyl nonanoate	1873	6561-39-3 	No Europe: ND USA: ND Japan: 10	See note 1	No safety concern
Hexyl decanoate	1874	10448-26-7	No Europe: ND USA: ND Japan: 0.3	See note 1	No safety concern

					
Heptyl heptanoate	1875	624-09-9 	No Europe: ND USA: ND Japan: 0.04	See note 1	No safety concern
Dodecyl propionate	1876	6221-93-8 	No Europe: 17 USA: ND Japan: 15	See note 1	No safety concern
Dodecyl butyrate	1877	3724-61-6 	No Europe: ND USA: ND Japan: 3	See note 1	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Sixty-six flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *132*).

[b] *Step 1:* All seven flavouring agents in this group are in structural class I (*2*).

[c] *Step 2:* All of the flavouring agents in this group are expected to be metabolized to innocuous products.

[d] The threshold for human intake for structural class I is 1800 µg/day. All intake values are expressed in µg/day.

Notes:

1. The ester is expected to undergo hydrolysis to the corresponding primary alcohol and carboxylic acid. The primary alcohol is oxidized to the corresponding aldehyde and carboxylic acid, which is completely metabolized in the fatty acid and tricarboxylic acid pathways to carbon dioxide and water.

Table 11

Annual volumes of production of esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Intake[b]		Annual volume from natural occurrence in foods (kg)[c]	Consumption ratio[d]
		µg/day	µg/kg bw per day		
Methyl hexanoate (1871)					
Europe	2193	235	4		
USA	336	41	1	1864.6	6
Japan	693	182	3		
Hexyl heptanoate (1872)					
Europe	ND	ND	ND		
USA	ND	ND	ND	+	NA
Japan	0.1	0.04	0.001		
Hexyl nonanoate (1873)					
Europe	ND	ND	ND		
USA	ND	ND	ND	−	NA
Japan	39	10	0.2		
Hexyl decanoate (1874)					
Europe	ND	ND	ND		
USA	ND	ND	ND	+	NA
Japan	1	0.3	0.004		
Heptyl heptanoate (1875)					
Europe	ND	ND	ND		
USA	ND	ND	ND	−	NA
Japan	0.2	0.04	0.001		
Dodecyl propionate (1876)					
Europe	141	17	0.3		
USA	ND	ND	ND	−	NA
Japan	56	15	0.2		
Dodecyl butyrate (1877)					
Europe	ND	ND	ND		
USA	ND	ND	ND	−	NA
Japan	12	3	0.1		
Total					
Europe	2334				
USA	336				
Japan	801				

NA, not available; ND, no intake data reported; +, reported to occur naturally in foods (*17*), but no quantitative data; −, not reported to occur naturally in foods.

[a] From references *14*, *15* and *16*. Total poundage values of <0.1 kg reported in the surveys (*14*, *15*, *16*) have been truncated to one place following the decimal point (0.1 kg).

[b] Intake (µg/person per day) calculated as follows: [(annual volume, kg) × (1×10^9 µg/kg)]/[population × survey correction factor × 365 days], where population (10%, "consumers only") = 32×10^6 for Europe, 28×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for

the surveys by the USA, Europe and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*14*, *15*, *16*).
Intake (μg/kg bw per day) calculated as follows: (μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

[c] Quantitative data for the USA reported by Stofberg & Grundschober (*18*).

[d] The consumption ratio is calculated as follows: (annual consumption from food, kg)/(most recent reported volume as a flavouring substance, kg).

Absorption, distribution, metabolism and elimination

In general, esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids are anticipated to be hydrolysed to their component alcohols and carboxylic acids (*22*). After hydrolysis, the component alcohols in this group of esters are all anticipated to be oxidized via their corresponding aldehydes to their carboxylic acids, which are then metabolized in the fatty acid β-oxidation pathway and the tricarboxylic acid pathway to carbon dioxide and water (*23*).

Application of the Procedure for the Safety Evaluation of Flavouring Substances

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to this group of flavouring agents, the Committee assigned all (Nos 1871–1877) to structural class I (*2*).

Step 2. All of the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all of the flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of all seven of the flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). According to the Procedure, the safety of these seven flavouring agents raises no concern when they are used at their current estimated levels of intake.

Table 10 summarizes the evaluations of the seven esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids in this group.

Consideration of combined intakes from use as flavouring agents

The common metabolites of these flavouring agents are linear alkyl carboxylic acids and alcohols of low toxic potential. In consequence, there would be no safety concerns in the unlikely event that the flavouring agents were to be consumed concurrently on a daily basis.

Consideration of secondary components

No flavouring agents in this group have minimum assay values of less than 95%.

Conclusion

In the previous evaluation of substances in this group, studies of acute toxicity, short-term and long-term studies of toxicity, and studies of genotoxicity and developmental toxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation.

The Committee concluded that these additional seven flavouring agents to the group of esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids evaluated previously would not give rise to safety concerns at the current estimated levels of intake.

No addendum to the toxicological monograph was prepared.

4.1.6 *Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers*

Explanation

At its sixty-fifth meeting (Annex 1, reference *177*), the Committee reviewed a group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers. The Committee at that meeting took note of the extensive evidence for the genotoxicity of several members of this group of flavouring agents related to furan, including the clastogenicity of 2-furyl methyl ketone (No. 1503) in mouse bone marrow. This substance accounts for 87–96% of total exposure to this group of flavouring agents. Noting also that furan is carcinogenic and is known to undergo epoxidation and ring opening to form a reactive 2-ene-1,4-dicarbonyl intermediate, the Committee at its sixty-fifth meeting expressed concern that the observed genotoxicity might be due to formation of a reactive metabolite. Few data on genotoxicity in vivo were available, and specific assays to address potential carcinogenicity in vivo were lacking. The Committee at its sixty-fifth meeting therefore concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group because of the above concerns. It was also concluded that studies of metabolism and in vivo assays for DNA reactivity, mutagenicity and carcinogenic potential of members of this group with representative structures would assist in resolving the concerns (Annex 1, reference *177*).

Additional studies of genotoxicity in vitro and in vivo with 2-furyl methyl ketone (No. 1503) were available to the Committee at its present meeting. The Committee included the new studies in its re-evaluation of the group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers.

Comments

As stated above, the main concern with this group arises primarily from the carcinogenicity of furan itself, which is believed to involve a reactive genotoxic metabolite formed by epoxidation and opening of the furan ring. Furan is not a member of this group of flavouring agents, but all the members of the group contain a furan ring with either one or two substituents of varying complexity. In some flavouring agents, a substituent is present on one side of the furan ring only, whereas in others, substituents are present on both sides. The presence of an extended side-chain attached to the furan ring would reduce the potential for epoxidation of the double bond and provide a site for detoxication via metabolism and elimination. The flavouring agent that has the simplest structure and would be predicted to have the greatest potential for ring oxidation is 2-methylfuran (No. 1487); there is evidence from studies in vitro and in vivo that this compound undergoes bioactivation to a reactive ring-opened metabolite that binds covalently to both protein and DNA. Data are not available on the influence of the nature and position of the ring substitution on potential for metabolic activation and adduct formation. After administration of a single dose, 2-methylfuran produced liver toxicity in rats from 50 mg/kg bw, but hepatotoxicity has not been reported for other members of this group in more extensive studies.

Testing for genotoxicity has been performed on eight members of this group of flavouring agents. The results of the studies of genotoxicity/mutagenicity in vitro that were already available to the Committee at its previous meeting were both positive and negative, with most positive results reported for chromosomal aberration. These, however, were less frequent in the presence of metabolic activation, indicating possible metabolic detoxication rather than bioactivation. 2-Methylfuran (No. 1487), for example, produced chromosomal aberrations in vitro, but the clastogenic activity was lower in the presence of a metabolizing system. The limited data available on genotoxicity in vivo showed no evidence of chromosomal aberration in mouse bone marrow or spermatocytes for 2-methylfuran. 2-Furyl methyl ketone (No. 1503) also induced no chromosomal aberrations in mouse spermatocytes, but a weak, transient increase in chromosomal aberrations was observed in mouse bone marrow, associated with mitodepression. *O*-Ethyl *S*-furfurylthiocarbonate (No. 1526) appeared not to induce micronucleus formation in mouse bone marrow.

The new data on 2-furyl methyl ketone (No. 1503) available to the Committee at its present meeting were a study on unscheduled DNA synthesis in cultured hepatocytes in vitro, a study on unscheduled DNA synthesis in rat liver in vivo/in vitro and a test for sister chromatid exchanges (SCEs) in mouse bone marrow in vivo. 2-Furyl methyl ketone did not induce unscheduled DNA synthesis either in vitro or in vivo/in vitro. However, it did induce SCEs, confirming the concern for clastogenicity as expressed by the Committee at its previous meeting. The Committee at its present meeting therefore considered that the new data available did not resolve the concerns expressed previously.

Evaluation

The Committee concluded that the Procedure could not be applied to this group because of the unresolved toxicological concerns. Studies that would assist in the safety evaluation include investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group.

A toxicological monograph was prepared. The Committee noted that the previously published monograph in the WHO Food Additives Series, No. 56 (Annex 1, reference *179*), was published in error, is incorrect and is formally withdrawn.

4.1.7 *Hydroxy- and alkoxy-substituted benzyl derivatives: additional compounds*

The Committee evaluated a group of six hydroxy- and alkoxy-substituted benzyl derivatives, including two vanillin acetals (Nos 1879 and 1882), one vanillin dimer (No. 1881), one alkoxy-hydroxylbenzaldehyde (No. 1878) and two alkoxybenzoyloxy derivatives (Nos 1880 and 1883). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee evaluated 46 other members of this group of flavouring agents at its fifty-seventh meeting (Annex 1, reference *154*). In addition, ethyl vanillin was evaluated at the eleventh meeting of the Committee (Annex 1, reference *14*), and a conditional ADI[1] of 0–10 mg/kg bw per day was assigned. At its thirty-fifth meeting (Annex 1, reference *88*), the Committee converted the conditional ADI to a temporary ADI of 0–5 mg/kg bw per day. At its

[1] "Conditional ADI", which signifies an ADI with special considerations, is a term no longer used by JECFA.

thirty-ninth meeting (Annex 1, reference *101*), the Committee extended the temporary ADI of 0–5 mg/kg bw per day. At its forty-fourth meeting (Annex 1, reference 116), the Committee established an ADI of 0–3 mg/kg bw per day. Vanillin was evaluated at the eleventh meeting of the Committee and assigned an ADI of 0–10 mg/kg bw per day. Methyl salicylate was evaluated at the eleventh meeting of the Committee, and an ADI of 0–0.5 mg/kg bw per day was assigned. Piperonal was evaluated at the eleventh meeting of the Committee and assigned an ADI of 0–2.5 mg/kg bw per day. All other members of this group were evaluated by the Procedure and concluded to be of no safety concern based on current estimated levels of intake.

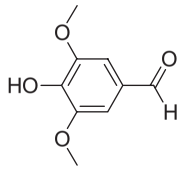
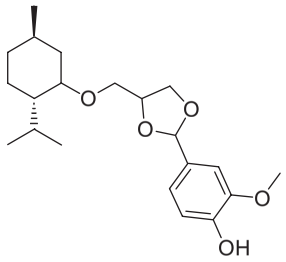
Two of the six flavouring agents in this group are natural components of food (Nos 1878 and 1881). They have been detected in a variety of fruits, types of honey and alcoholic beverages, but quantitative data on natural occurrence were not available.

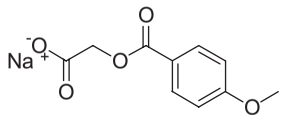
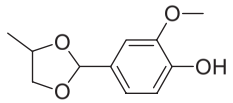
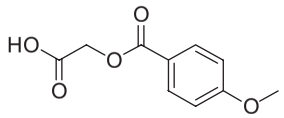
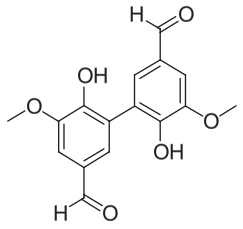
Assessment of dietary exposure

The total annual production volume of the six hydroxy- and alkoxy-substituted benzyl derivatives is approximately 822 kg in Europe, 61 kg in the USA and 204 kg in Japan. More than 99% of the annual production volume in Europe and Japan is accounted for by vanillin propylene glycol acetal (No. 1882). More than 85% of the annual production volume in the USA is accounted for by sodium 4-methoxybenzoyloxyacetate (No. 1880) and 4-methoxybenzoyloxyacetic acid (No. 1883). The daily per capita intake of each flavouring agent is reported in Table 12.

Table 1

Summary of results of safety evaluations of hydroxy- and alkoxy-substituted benzyl derivatives used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class I					
4-Hydroxy-3,5-dimethoxy benzaldehyde	1878	134-96-3 	No Europe: 0.01 USA: ND Japan: ND	See note 1	No safety concern
Vanillin 3-(*l*-menthoxy) propane-1,2-diol acetal	1879	180964-47-0 	No Europe: 0.01 USA: 0.4 Japan: ND	See note 2	No safety concern

Sodium 4-methoxybenzoyloxyacetate	1880	17114-82-8 	No Europe: 0.01 USA: 4 Japan: ND	See note 2	No safety concern
Vanillin propylene glycol acetal	1882	68527-74-2 	No Europe: 88 USA: 0.5 Japan: 54	See note 2	No safety concern
4-Methoxybenzoyloxyacetic acid	1883	10414-68-3 	No Europe: 0.01 USA: 2.2 Japan: ND	See note 2	No safety concern
Structural class III					
Divanillin	1881	2092-49-1 	No Europe: 0.01 USA: 0.2 Japan: ND	See note 1	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Forty-six flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *154*).

[b] *Step 1:* Five flavouring agents (Nos 1878-1880, 1982 and 1983) were assigned to structural class I, and the remaining flavouring agent (No. 1881) was assigned to structural class III.

[c] *Step 2:* All the agents in this group are expected to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I and III are 1800 and 90 µg/day, respectively. All intake values are expressed in µg/day. The combined per capita intakes of the five flavouring agents with the highest volume with the common metabolite 4-hydroxy-3-methoxy benzoic acid were 60 826, 166 140 and 0.3 µg/person per day in Europe, the USA and Japan for all agents in the group, including the six agents evaluated at the present meeting and those evaluated previously.

Notes:

1. Detoxication by excretion in the urine unchanged or as glucuronic acid, glycine or sulfate conjugates; aldehyde groups undergo oxidation or reduction to the corresponding carboxylic acid or alcohol, respectively, followed by conjugation and excretion; *O*-dealkylation followed by decarboxylation and reduction of benzyl groups to the methyl analogue.
2. Detoxication as in note 1 plus hydrolysis of esters to corresponding benzoic acid derivatives or acetal hydrolysis to the parent benzaldehyde derivative and simple aliphatic alcohol.

Absorption, distribution, metabolism and elimination

The hydrolysis of aromatic acetals in simulated gastric juice and intestinal fluid supports the conclusion that the acetal functional group is hydrolysed before absorption in vivo. Both vanillin 3-(*l*-menthoxy)propane-1,2-diol acetal (No. 1879) and vanillin propylene glycol acetal (No. 1882) undergo hydrolysis under acidic conditions to form the corresponding alcohol and aldehyde, which will be rapidly metabolized and eliminated. The resulting hydroxy- and alkoxy-substituted derivatives are rapidly absorbed from the intestine, metabolized in the liver and excreted unchanged or as sulfate or glucuronide conjugates. Minor metabolic pathways include *O*-demethylation, reduction and/or decarboxylation.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned five flavouring agents (Nos 1878–1880, 1882 and 1883) to structural class I. The Committee assigned the remaining flavouring agent (No. 1881) to structural class III.

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all of the flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of the five flavouring agents in structural class I are below the threshold of concern (i.e. 1800 µg/person per day for class I). The estimated daily per capita intake for the flavouring agent in structural class III is below the threshold of concern (i.e. 90 µg/person per day for class III). According to the Procedure, these six flavouring agents raise no safety concerns when they are used at the current estimated levels of intake.

Table 12 summarizes the evaluations of the six hydroxy- and alkoxy-substituted benzyl derivatives (Nos 1878–1883) in this group.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are metabolized to a common metabolite, 4-hydroxy-3-methoxy benzoic acid (No. 959), in structural class I. For this common metabolite, the five flavouring agents with the highest intakes, considered in this and previous evaluations, correspond to Nos 889, 1882, 891, 959 and 886. In the unlikely event that these five flavouring agents were to be consumed concurrently on a daily basis, the estimated combined

intake[1] of 60 826 and 166 140 µg/person per day in Europe and the USA, respectively, would exceed the threshold of concern (i.e. 1800 µg/person per day for class I). However, these five flavouring agents, as well as the other 47 flavouring agents evaluated previously and currently, are all expected to be metabolized efficiently, and the available metabolic pathways would not be saturated. Moreover, more than 90% of the potential combined intakes in both Europe and the USA are accounted for by vanillin (No. 889), for which the Committee had maintained the ADI of 0–10 mg/kg bw at its fifty-seventh meeting (Annex 1, reference *154*). The Committee noted that the potential combined intakes do not exceed this ADI. Overall, the evaluation of the data indicated that combined intake would not raise safety concerns.

Consideration of secondary components

The three flavouring agents of this group that are derivatives of vanillin (Nos 1879, 1881 and 1882) have minimum assay values of less than 95%. However, the major secondary component in each of these is vanillin (No. 889), for which an ADI of 0–10 mg/kg bw per day has been allocated (Annex 1, reference *14*) and which the Committee concluded was of no safety concern at current estimated levels of intake as a flavouring agent. Information on the safety of the secondary component of this compound is summarized in Annex 4 (Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%).

Conclusion

In the previous evaluations of substances in this group, studies of acute toxicity, short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, and studies of genotoxicity and reproductive toxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluations (Annex 1, reference *154*).

The Committee concluded that these six flavouring agents, which are additions to the group of hydroxy- and alkoxy-substituted benzyl derivatives evaluated previously, would not give rise to safety concerns at the current estimated levels of intake.

An addendum to the toxicological monograph was prepared.

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[1] Combined intake was calculated on a molar basis relative to the formation of a common metabolite. In this instance, the common metabolite is 4-hydroxy-3-methoxy benzoic acid, with a relative molecular mass of 168.15.

4.1.8 *Miscellaneous nitrogen-containing substances: additional compounds*

The Committee evaluated a group of 14 flavouring agents that includes 11 alkyl isothiocyanates (Nos 1884–1891 and 1893–1895) and 3 mercapto-isothiocyanates (Nos 1892, 1896 and 1897). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents established at the forty-ninth meeting (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee evaluated 16 other members of this group of flavouring agents at its sixty-fifth meeting (Annex 1, reference *177*). All the substances were concluded to be of no safety concern based on current estimated levels of intake; however, the evaluation was conditional for 10 of these substances, because the estimated exposure was based on anticipated annual volumes of production. At the present meeting, actual volumes of production for these substances were provided, and the Committee confirmed that these 10 substances were of no safety concern based on current estimated levels of intake (see section 4.2).

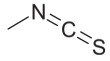
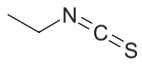
Twelve of the 14 substances (Nos 1884–1890, 1892–1894, 1896 and 1897) have been reported to occur naturally in foods. They have been detected in cabbages, radish, mustards, wasabi, garlic, bread, milk and wines.

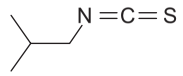
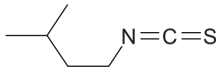
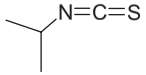
Assessment of dietary exposure

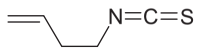
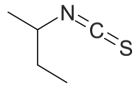
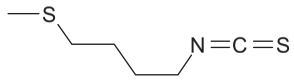
The total annual volume of production of the 14 flavouring agents in this group is approximately 913 kg in Japan. More than 54% of the total annual volume of production in Japan is accounted for by a single substance in this group — namely, 4-pentenyl isothiocyanate (No. 1893), which has an estimated per capita intake of 131 μg/person per day. More than 37% of the total annual volume of production is accounted for by two other substances in this group — namely, 3-butenyl isothiocyanate (No. 1889) and 5-hexenyl isothiocyanate (No. 1894), which have estimated per capita intakes of 50 and 40 μg/person per day, respectively. The estimated per capita intakes of all the other flavouring agents in the group range from 0.03 to 12 μg/person per day, with most of the intake values at the lower end of this range. The estimated per capita intake of each flavouring agent is reported in Table 13.

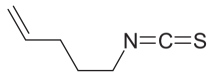
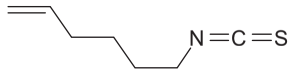
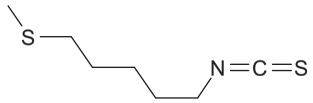
Table 13

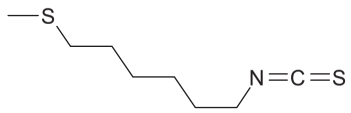
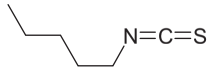
Summary of results of safety evaluations of miscellaneous nitrogen-containing substances used as flavouring agents[a,b,c]

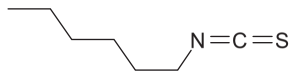
Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class II						
Methyl isothiocyanate	1884	556-61-6 	No Europe: ND USA: ND Japan: 0.03	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 24 million times the estimated daily intake of methyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
Ethyl isothiocyanate	1885	542-85-8 	No Europe: ND USA: ND Japan: 0.03	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 24 million times the estimated daily intake of ethyl isothiocyanate when	See note 1	No safety concern

				used as a flavouring agent.		
Isobutyl isothiocyanate	1886	591-82-2 	No Europe: ND USA: ND Japan: 3	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 240 000 times the estimated daily intake of isobutyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
Isoamyl isothiocyanate	1887	628-03-5 	No Europe: USA: Japan: 0.03	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 24 million times the estimated daily intake of isoamyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
Isopropyl isothiocyanate	1888	2253-73-8 	No Europe: ND USA: ND Japan: 1	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 600 000 times the estimated daily intake of isopropyl isothiocyanate when	See note 1	No safety concern

				used as a flavouring agent.		
3-Butenyl isothiocyanate	1889	3386-97-8 	No Europe: ND USA: ND Japan: 50	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 15 000 times the estimated daily intake of 3-butenyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
2-Butyl isothiocyanate	1890	4426-79-3 	No Europe: ND USA: ND Japan: 12	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 60 000 times the estimated daily intake of 2-butyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
4-(Methylthio)butyl isothiocyanate	1892	4430-36-8 	No Europe: ND USA: ND Japan: 0.1	Yes. The NOEL[e] of 30 mg/kg bw per day for the related substance 3-(methylthio)pr yl isothiocyanate (No. 1564) is 15 million times the estimated daily intake of	See note 1	No safety concern

				4-(methylthio)butyl isothiocyanate when used as a flavouring agent.		
4-Pentenyl isothiocyanate	1893	18060-79-2 	No Europe: ND USA: ND Japan: 132	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 5400 times the estimated daily intake of 4-pentenyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
5-Hexenyl isothiocyanate	1894	49776-81-0 	No Europe: ND USA: ND Japan: 40	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 17 000 times the estimated daily intake of 5-hexenyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
5-(Methylthio)pentyl isothiocyanate	1896	4430-42-6 	No Europe: ND USA: ND Japan: 0.1	Yes. The NOEL[e] of 30 mg/kg bw per day for the related substance 3-(methylthio)propyl isothiocyanate	See note 1	No safety concern

				(No. 1564) is 15 million times the estimated daily intake of 5-(methylthio)pentyl isothiocyanate when used as a flavouring agent.		
6-(Methylthio)hexyl isothiocyanate	1897	4430-39-1 	No Europe: ND USA: ND Japan: 3	Yes. The NOEL[e] of 30 mg/kg bw per day for the related substance 3-(methylthio)propyl isothiocyanate (No. 1564) is 600 000 times the estimated daily intake of 6-(methylthio)hexyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern
Structural class III						
Amyl isothiocyanate	1891	629-12-9 	No Europe: ND USA: ND Japan: 0.03	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 24 million times the estimated daily intake of amyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern

Hexyl isothiocyanate	1895	4404-45-9 	No Europe: ND USA: ND Japan: 0.8	Yes. The NOEL[e] of 12 mg/kg bw per day for the related substance allyl isothiocyanate (No. 1560) is 1.2 million times the estimated daily intake of hexyl isothiocyanate when used as a flavouring agent.	See note 1	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Sixteen flavouring agents in this group were previously evaluated by the Committee at its sixty-fifth meeting (Annex 1, reference *177*).

[b] *Step 1:* Twelve of the flavouring agents (Nos 1884-1890, 1892-1894, 1896 and 1897) in this group were assigned to structural class II, and the remaining two flavouring agents (Nos 1891 and 1895) were assigned to structural class III.

[c] *Step 2:* None of the flavouring agents in this group can be predicted to be metabolized to innocuous products. In addition, there were toxicological concerns associated with these substances.

[d] The thresholds of concern for structural classes II and III are 540 and 90 µg/day, respectively. All intake values are expressed in µg/day. The combined per capita intakes of the five flavouring agents with the highest volume in a homologous series of linear saturated isothiocyanates in structural classes II and III are 2 and 0.89 µg/person per day in Europe and Japan, respectively. The combined per capita intake of the four flavouring agents with the highest volume in a homologous series of branched-chain saturated isothiocyanates in structural class II is 16 µg/person per day in Japan. The combined per capita intakes of the four flavouring agents with the highest volume in a homologous series of linear saturated mercapto-isothiocyanates in structural class II are 13, 52 and 3.2 µg/person per day in Europe, the USA and Japan, respectively. The combined per capita intakes of the four flavouring agents with the highest volume in a homologous series of linear unsaturated isothiocyanates in structural class II are 1502, 133 and 222 µg/person per day in Europe, the USA and Japan, respectively.

[e] According to the decision taken by the Committee at its sixty-eighth meeting (Annex 1, reference *187*), this would now be termed a NOAEL.

Notes:

1. Rapidly absorbed, principally conjugated with glutathione and excreted in the urine.

Absorption, distribution, metabolism and elimination

Isothiocyanates are readily absorbed and distributed to all major tissues in studies in rodents. Peak concentrations in these tissues are achieved between 2 and 8 h after dosing. Metabolic studies in humans, mice and rats indicate that isothiocyanates react readily with reduced glutathione (GSH) to form a conjugate as the primary metabolite and that the reaction is catalysed enzymatically by glutathione *S*-transferase, although a slower non-enzymatic reaction can also occur. Both reactions occur in a pH-dependent equilibrium. In rats, the *N*-acetylcysteine conjugates appear as the major metabolite in urine, whereas some isothiocyanate-GSH conjugates are excreted into bile.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned 12 (Nos 1884–1890, 1892–1894, 1896 and 1897) to structural class II and the remaining 2 (Nos 1891 and 1895) to structural class III.

Step 2. Although conjugation with GSH is the major pathway of metabolism for alkyl isothiocyanates, a significant proportion of the excreted metabolites were not identified in studies in animals. Therefore, none of the flavouring agents in this group can be predicted to be metabolized to innocuous products. In addition, because of toxicological concerns (possible effects on the urinary bladder), the evaluation of these 14 flavouring agents proceeded via the B-side of the Procedure.

Step B3. The estimated daily per capita intakes of all 12 of the flavouring agents in structural class II and of both of the flavouring agents in structural class III are below the thresholds of concern for their class (i.e. 540 µg/day for class II; 90 µg/day for class III). Therefore, the evaluation of all 14 flavouring agents in the group proceeded to step B4.

Step B4. For methyl isothiocyanate (No. 1884), ethyl isothiocyanate (No. 1885), isobutyl isothiocyanate (No. 1886), isoamyl isothiocyanate (No. 1887), isopropyl isothiocyanate (No. 1888), 3-butenyl isothiocyanate (No. 1889), 2-butyl isothiocyanate (No. 1890), amyl isothiocyanate (No. 1891), 4-pentenyl isothiocyanate (No. 1893), 5-hexenyl isothiocyanate (No. 1894) and hexyl isothiocyanate (No. 1895), the NOEL[1] of 12 mg/kg bw per day for the structurally related flavouring agent allyl isothiocyanate (No. 1560) from a 2-year study in rats and mice treated via gavage is appropriate because they are all alkyl isothiocyanates and will be metabolized via

[1] According to the decision taken by the Committee at its sixty-eighth meeting (Annex 1, reference *187*), this would now be termed a NOAEL.

similar metabolic pathways. The NOEL of 12 mg/kg bw per day for allyl isothiocyanate (No. 1560) provides margins of safety in the range of 5400–24 000 000 in relation to their estimated levels of intake.

For 4-(methylthio)butyl isothiocyanate (No. 1892), 5-(methylthio)pentyl isothiocyanate (No. 1896) and 6-(methylthio)hexyl isothiocyanate (No. 1897), the NOEL of 30 mg/kg bw per day for the structurally related flavouring agent 3-(methylthio)propyl isothiocyanate (No. 1564) from an 84-day feeding study in rats is appropriate because they are all linear mercapto-isothiocyanates and will be metabolized via similar metabolic pathways. The NOEL of 30 mg/kg bw per day for 3-(methylthio)propyl isothiocyanate (No. 1564) provides margins of safety in the range of 600 000–15 000 000 in relation to their estimated levels of intake.

Table 13 summarizes the evaluations of the 14 miscellaneous nitrogen-containing substances in this group.

Consideration of combined intakes from use as flavouring agents

In the unlikely event that the flavouring agents in a homologous series of linear saturated isothiocyanates in structural classes II and III, of which the highest intakes correspond to Nos 1561, 1884, 1885, 1891 and 1895 in Europe and Japan, were to be consumed concurrently on a daily basis, the estimated combined intakes of 2 and 0.89 μg/person per day in Europe and Japan, respectively, would not exceed the threshold of concern (i.e. 540 μg/person per day for class II and 90 μg/person per day for class III).

In the unlikely event that the flavouring agents in a homologous series of branched-chain saturated isothiocyanates in structural class II, of which the highest intakes correspond to Nos 1886–1888 and 1890 in Japan, were to be consumed concurrently on a daily basis, the estimated combined intake of 16 μg/person per day in Japan would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

In the unlikely event that the flavouring agents in a homologous series of linear saturated mercapto-isothiocyanates in structural class II, of which the highest intakes correspond to Nos 1564, 1892, 1896 and 1897 in Europe, the USA and Japan, were to be consumed concurrently on a daily basis, the estimated combined intakes of 13, 52 and 3.2 μg/person per day in Europe, the USA and Japan, respectively, would not exceed the threshold of concern (i.e. 540 μg/person per day for class II).

In the unlikely event that the flavouring agents in a homologous series of linear unsaturated isothiocyanates in structural class II, of which the highest intakes correspond to Nos 1560, 1889, 1893 and 1894 in Europe, the USA and Japan, were to be consumed concurrently on a daily basis, the estimated

combined intakes would be 1502, 133 and 222 μg/person per day in Europe, the USA and Japan, respectively. The estimate combined intake would exceed the threshold of concern in Europe (i.e. 540 μg/person per day for class II). The intake in Europe, however, is due mainly to allyl isothiocyanate (No. 1560). Allyl isothiocyanate has a NOEL of 12 mg/kg bw per day in 2-year studies in rats and mice, which provides a margin of safety of 480 in relation to the estimated level of intake. The overall evaluation of the data indicates that combined intake would not raise safety concerns.

Consideration of secondary components

No flavouring agents in this group have minimum assay values of less than 95%.

Conclusion

In the previous evaluation of substances in this group, studies of acute toxicity, short-term and long-term studies of toxicity and studies of genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation.

The Committee concluded that these 14 flavouring agents, which are additions to the group of miscellaneous nitrogen-containing substances evaluated previously, do not give rise to safety concerns at the current estimated levels of intake.

An addendum to the toxicological monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.9 *Monocyclic and bicyclic secondary alcohols, ketones and related esters: additional compounds*

The Committee evaluated a group of nine monocyclic and bicyclic secondary alcohols, ketones and related esters, including three bicyclic ketones (Nos 1862, 1868 and 1870), two secondary bicyclic alcohols (Nos 1865 and 1866) and four esters of bicyclic secondary alcohols (Nos 1863, 1864, 1867 and 1869). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

The Committee evaluated 32 other members of this group of flavouring agents at its sixty-third meeting (Annex 1, reference *173*) and concluded that all 32 members were of no safety concern based on current estimated levels of intake. However, for several of the flavouring agents in this group, that evaluation was based on anticipated production volumes. Actual production

volumes were subsequently provided and were used for the re-evaluation of these flavouring agents at the present meeting. The only member of this group for which the actual production volume resulted in an estimated intake that exceeded the TTC was L-monomenthyl glutarate (No. 1414), which was re-evaluated at the current meeting and found not to present a safety concern (see section 4.2).

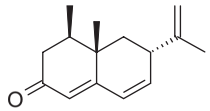
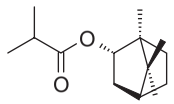
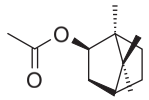
Four of the nine flavouring agents in this group are natural components of foods (Nos 1862, 1865, 1868 and 1870). Quantitative data on these flavouring agents were not reported, but they have been detected in a wide variety of fruits, including lingon berries, blackberries, raspberries, strawberries, plum, melon, apricot, kiwi, mango and cherimoya, and a variety of herbs and spices, including ginger, scotch spearmint oil, saffron, thyme, rosemary, lemon balm, eucalyptus oil and mastic gum oil (*17*).

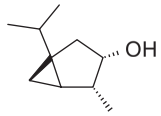
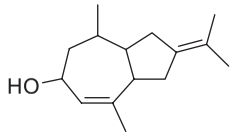
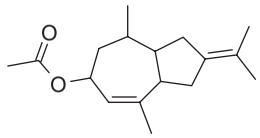
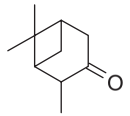
Assessment of dietary exposure

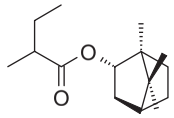
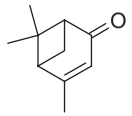
The total annual volume of production of the nine monocyclic and bicyclic secondary alcohols, ketones and related esters is approximately 769 kg in Europe, 484 kg in the USA and 0.5 kg in Japan (*14*, *15*, *16*). Approximately 96% of the total annual volume of production in Europe is accounted for by *l*-bornyl acetate (No. 1864), and approximately 97% of the total annual volume in the USA is accounted for by vetiveryl acetate (No. 1867) and verbenone (No. 1870). The daily per capita intake of each agent is reported in Table 14. Annual volumes of production of this group of flavouring agents are summarized in Table 15.

Table 14

Summary of results of safety evaluations of monocyclic and bicyclic secondary alcohols, ketones and related esters used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class I					
Dehydronootkatone	1862	5090-63-1 	No Europe: 0.01 USA: ND Japan: ND	See notes 2, 3, 4 and 5	No safety concern
Isobornyl isobutyrate	1863	85586-67-0 	No Europe: 0.01 USA: ND Japan: ND	See notes 1 and 2	No safety concern
l-Bornyl acetate	1864	5655-61-8 	No Europe: 80 USA: 1 Japan: ND	See notes 1 and 2	No safety concern

Thujyl alcohol	1865	21653-20-3 	No Europe: 0.01 USA: 0.2 Japan: 0.03	See notes 2 and 4	No safety concern
Structural class II					
Vetiverol	1866	89-88-3 	No Europe: 2 USA: ND Japan: ND	See notes 2, 4 and 5	No safety concern
Vetiveryl acetate	1867	117-98-6 	No Europe: 0.01 USA: 29 Japan: 0.03	See notes 1, 2, 4 and 5	No safety concern
3-Pinanone	1868	18358-53-7 	No Europe: 0.01 USA: ND Japan: ND	See notes 2 and 4	No safety concern

Isobornyl 2-methylbutyrate	1869	94200-10-9 	No Europe: 0.01 USA: ND Japan: ND	See notes 1 and 2	No safety concern
Verbenone	1870	80-57-9 	No Europe: 1 USA: 29 Japan: 0.09	See notes 2, 3 and 4	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Thirty-two flavouring agents in this group were previously evaluated by the Committee at its sixty-third meeting (Annex 1, reference *173*).

[b] *Step 1:* Four flavouring agents (Nos 1862-1865) are in structural class I, and five (Nos 1866-1870) are in structural class II.

[c] *Step 2:* All the flavouring agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I and II are 1800 and 540 µg/day, respectively. All intake values are expressed in µg/day. The combined per capita intakes of the five flavouring agents with the highest volume with the common metabolite, isoborneol, in structural class I, is 843 µg/day in Europe. The combined per capita intakes of the five highest volume flavouring agents with the common metabolite, borneol, in structural class I, are 232 and 35 µg/day in Europe and the USA, respectively.

Notes:

1. Ester hydrolysis to liberate the corresponding alcohol and carboxylic acid.
2. Formation of glucuronic acid conjugates, which are subsequently excreted in the urine.
3. Reduction to yield the corresponding alcohol.
4. Hydroxylation of alkyl ring substituents and ring positions.
5. Oxidation and hydration of exocyclic and, to a lesser extent, endocyclic double bonds.

Table 15

Annual volumes of production of monocyclic and bicyclic secondary alcohols, ketones and related esters used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Intake[b]	
		µg/day	µg/kg bw per day
Dehydronootkatone (1862)			
Europe	0.1	0.01	0.0002
USA	ND	ND	ND
Japan	ND	ND	ND
Isobornyl isobutyrate (1863)			
Europe	0.1	0.01	0.0002
USA	ND	ND	ND
Japan	ND	ND	ND
l-Bornyl acetate (1864)			
Europe	744	80	1
USA	8	1	0.02
Japan	ND	ND	ND
Thujyl alcohol (1865)			
Europe	0.1	0.01	0.0002
USA	2	0.2	0.003
Japan	0.1	0.03	0.0004
Vetiverol (1866)			
Europe	14	2	0.03
USA	ND	ND	ND
Japan	ND	ND	ND
Vetiveryl acetate (1867)			
Europe	0.1	0.01	0.0002
USA	233	29	0.5
Japan	0.1	0.03	0.0004
3-Pinanone (1868)			
Europe	0.1	0.01	0.0002
USA	ND	ND	ND
Japan	ND	ND	ND
Isobornyl 2-methylbutyrate (1869)			
Europe	0.1	0.01	0.0002
USA	ND	ND	ND
Japan	ND	ND	ND
Verbenone (1870)			
Europe	10	1	0.02
USA	241	29	0.5
Japan	0.3	0.09	0.001
Total			
Europe	769		
USA	484		
Japan	0.5		

ND, no intake data reported.

[a] From references *14*, *15* and *16*. Total poundage values of <0.1 kg reported in the surveys (*14*, *15*, *16*) have been truncated to one place following the decimal point (0.1 kg).

[b] Intake (μg/person per day) calculated as follows: [(annual volume, kg) × (1×10^9 μg/kg)]/[population × survey correction factor × 365 days], where population (10%, "consumers only") = 32×10^6 for Europe, 28×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for the surveys by the USA, Europe and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*14*, *15*, *16*).
Intake (μg/kg bw per day) calculated as follows: (μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

Absorption, distribution, metabolism and elimination

Studies in humans, dogs and rabbits have shown that monocyclic and bicyclic secondary alcohols and ketones of this group are rapidly absorbed, distributed, metabolized and excreted, mainly in the urine. Small amounts may be expired in exhaled air. The esters within this group are expected to be hydrolysed in humans to their component secondary alcohol and carboxylic acid.

The major metabolic pathway of the ketones involves reduction to the corresponding secondary alcohols, which are subsequently excreted primarily as the glucuronic acid conjugates (Annex 1, reference *173*). In addition to reductive pathways, alicyclic ketones and, to a lesser extent, secondary alcohols containing an alkyl side-chain undergo oxidation of the side-chain to form polar poly-oxygenated metabolites that are excreted either unchanged or as the glucuronide or sulfate conjugates, mainly in the urine.

For more lipophilic ketones or those with sterically hindered functional groups, oxidation of a ring position or aliphatic side-chain by CYP may compete with reduction of the ketone functional group or oxidation of the alcohol functional group (*24*, *25*). For example, bicyclic ketones tend to show greater lipophilicity and steric hindrance of the carbonyl function than do short-chain aliphatic or monocyclic ketones, which are primarily reduced to the corresponding secondary alcohol. As such, bicyclic ketones are expected to be poor substrates for cytosolic reducing enzymes. Consequently, the predominant detoxication route is CYP-mediated hydroxylation to yield polar, excretable poly-oxygenated metabolites. Recent reports by Miyazawa et al. (*26*, *27*) demonstrate that (−)-verbenone (No. 1870) undergoes CYP-mediated hydroxylation to form (−)-10-hydroxyverbenone (hydroxylation of the methyl substituent). This reaction occurs in microsomes of the liver of male rats and in human liver. The calculated metabolic clearance in vitro (maximum rate [V_{max}]/Michaelis-Menten constant [K_m]) for this reaction was similar for microsomes of human liver and untreated microsomes of the liver of male rats. Recombinant human CYP2A6 and CYP2B6 demonstrated in

vitro metabolic clearance values similar to those for recombinant CYP2B1 and CYP2C11 from rats.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned four of the flavouring agents (Nos 1862–1865) to structural class I (*2*). The remaining five flavouring agents (Nos 1866– 1870) were assigned to structural class II (*2*).

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all of the flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of all four of the flavouring agents in structural class I are below the threshold of concern (i.e. 1800 µg/person per day for class I). The estimated daily per capita intakes of the five flavouring agents in structural class II are below the threshold of concern (540 µg/person per day for class II). According to the Procedure, the safety of these nine flavouring agents raises no concern when they are used at their current estimated levels of intake.

Table 14 summarizes the safety evaluations of the nine monocyclic and bicyclic secondary alcohols, ketones and related esters (Nos 1862–1870) in this group.

Toxicological data

Studies of acute toxicity with *l*-bornyl acetate (No. 1864) in male and female rats and with vetiveryl acetate (No. 1867) in rats (male) and mice (sex not specified) report LD_{50} values above 5000 mg/kg bw (*28*, *29*, *30*). These data and those previously evaluated by the Committee at its sixty-third meeting (Annex 1, reference *173*) demonstrate the low acute oral toxicity of these nine flavouring agents.

At its sixty-third meeting, the Committee reviewed the results of a 28-day study in rats (male and female) given nootkatone (No. 1398) or verbenone (No. 1870) at a dose of 10 mg/kg bw per day by oral gavage (*31*; Annex 1, reference *173*). At study termination, necropsies were perfomed and histopathological evaluations were conducted on selected tissues from all rats. No clinically observable signs of toxicity were reported. There were no adverse effects on body weight, survival, food consumption, water consumption, or haematological or blood chemistry parameters. Organ weights for the rats receiving nootkatone or verbenone were comparable with those of the

controls. No treatment-related macroscopic effects were reported. Histopathological examination revealed accumulation of globular eosinophilic material in the tubular epithelium of male rats treated with either verbenone or nootkatone at a dose of 10 mg/kg bw per day. The authors concluded and the Committee of the sixty-third meeting agreed that this finding is consistent with the presence of hyaline droplet nephropathy, which results from the excessive accumulation of alpha-2u-globulin in renal proximal tubular epithelial cells (Annex 1, reference *173*). Accumulation of alpha-2u-globulin in the proximal tubular epithelium is considered to be of no relevance to humans. There was no treatment-related difference in the distribution of the grades of severity of this condition between rats given verbenone and rats given nootkatone. The authors concluded that oral administration of verbenone to rats for 28 consecutive days at a single dose level of 10 mg/kg bw per day did not result in any toxicologically significant effects (*31*). On the basis of these results, the Committee at its sixty-third meeting identified a NOAEL of 10 mg/kg bw per day for nootkatone (Annex 1, reference *173*), and the Committee at its present meeting identified a NOAEL of 10 mg/kg bw per day for verbenone.

Consideration of combined intakes from use as flavouring agents[1]

In the unlikely event that the flavouring agents in structural class I with the common metabolite, isoborneol (No. 1386), considered in this and in previous evaluations, for which the five highest intakes correspond to Nos 1386, 1388, 1390, 1391 and 1394, were to be consumed concurrently on a daily basis, the estimated combined intake of 843 µg/person per day would not exceed the human intake threshold for structural class I, 1800 µg/person per day. In the unlikely event that the flavouring agents in structural class I with a common metabolite, borneol (No. 1385), considered in this and previous evaluations, for which the five highest intakes correspond to Nos 1385, 1864, 1387, 1389 and 1393 in Europe and Nos 1385, 1412, 1392, 1387 and 1864 in the USA, were to be consumed concurrently on a daily basis, the estimated combined intakes of 232 and 35 µg/person per day in Europe and the USA, respectively, would not exceed the human intake threshold for structural class I, 1800 µg/person per day. Overall evaluation of the data indicated that combined intake would not raise safety concerns.

Consideration of secondary components

All nine flavouring agents in this group have a minimum assay value of ≥95%. Hence, it is not necessary to consider secondary components.

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[1] Combined intake was calculated on a molar basis relative to the formation of a common metabolite.

Conclusion

In the previous evaluation of substances in this group, studies of acute toxicity, short-term studies of toxicity and studies of genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluation (Annex 1, reference *173*).

The Committee concluded that the addition of these nine flavouring agents to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters evaluated previously does not raise any safety concerns at the current estimated levels of intake.

No addendum to the toxicological monograph was prepared.

4.1.10 *Substances structurally related to menthol: additional compounds*

The Committee evaluated a group of 10 flavouring agents structurally related to menthol, including 4 esters of menthol (Nos 1852, 1854, 1855 and 1858), 1 ketone (No. 1856), 3 alicyclic alcohols or ethers (Nos 1853, 1860 and 1861), 1 diketone (No. 1857) and 1 ketal (No. 1859). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents established by the Committee at its forty-ninth meeting (Fig. 1) (Annex 1, reference *131*). None of these flavouring agents has previously been evaluated by the Committee.

At its fifty-first meeting, the Committee evaluated menthol and 13 other members of this group of flavouring agents (Annex 1, reference *137*). All 14 agents in that group were concluded to be of no safety concern based on current estimated levels of intake.

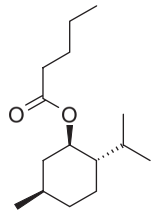
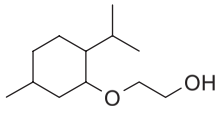
Five of the 10 additional flavouring agents in this group are natural components of foods (Nos 1852, 1860, 1856, 1857 and 1861). They have been detected in a variety of peppermint and cornmint oils, honeys, teas, starfruit, shrimp, lemon balm, citrus peel oils and cognac.

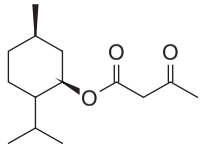
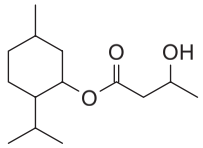
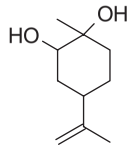
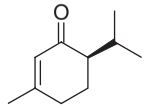
Assessment of dietary exposure

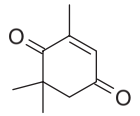
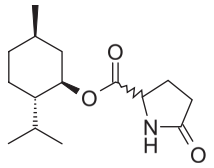
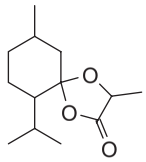
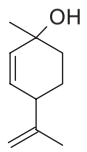
The total annual volume of production of the 10 substances structurally related to menthol is approximately 409 kg in Europe, 485 kg in the USA and 162 kg in Japan. The estimated daily per capita intake of each flavouring agent is reported in Table 16.

Table 16

Summary of results of safety evaluations of substances structurally related to menthol used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I					
Menthyl valerate	1852	89-47-4 	No Europe: 0.01 USA: ND Japan: 2	See notes 1 and 2	No safety concern
2-(*l*-Menthoxy)ethanol	1853	38618-23-4 	No Europe: 0.01 USA: 12 Japan: ND	See note 3	No safety concern

l-Menthyl acetoacetate	1854	59557-05-0 	No Europe: ND USA: 24 Japan: ND	See notes 1 and 2	No safety concern
l-Menthyl (*R*,*S*)-3-hydroxybutyrate	1855	108766-16-1 	No Europe: ND USA: ND Japan: 39	See notes 1 and 2	No safety concern
8-*p*-Menthene-1,2-diol	1860	1946-00-5 	No Europe: ND USA: ND Japan: 0.1	See note 3	No safety concern
Structural class II					
l-Piperitone	1856	4573-50-6 	No Europe: 0.01 USA: 17 Japan: ND	See note 4	No safety concern

2,6,6-Trimethylcyclohex-2-ene-1,4-dione	1857	1125-21-9 	No Europe: 44 USA: 3 Japan: 1	See note 4	No safety concern
Menthyl pyrrolidone carboxylate	1858	68127-22-0 	No Europe: 0.01 USA: 3 Japan: ND	See notes 1 and 2	No safety concern
'3,9-Dimethyl-6-(1-methylethyl)-1,4-dioxaspiro [4.5]decan-2-one	1859	831213-72-0 	No Europe: ND USA: 0.1 Japan: ND	See notes 1 and 4	No safety concern
d-2,8-*p*-Menthadien-1-ol	1861	22771-44-4 	No Europe: ND USA: ND Japan: 0.03	See note 3	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] Fourteen flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *137*).

[b] *Step 1:* Five of the flavouring agents (Nos 1852-1855 and 1860) were assigned to structural class I, and the remaining five flavouring agents (Nos 1856-1859 and 1861) were assigned to structural class II.

[c] *Step 2:* All of the agents in this group are expected to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I and II are 1800 and 540 µg/day, respectively. All intake values are expressed in µg/day. The combined per capita intakes of the five flavouring agents with the highest volume with the common metabolite, menthol, in structural class I are 1527 µg/day in Europe and 3513 µg/day in the USA.

Notes:

1. Anticipated to hydrolyse to their corresponding menthol derivative and carboxylic acid.
2. Menthol is primarily conjugated with glucuronic acid and excreted in the urine.
3. Anticipated to primarily conjugate with glucuronic acid and be excreted in the urine.
4. Anticipated to be reduced to the corresponding alcohol, primarily conjugate with glucuronic acid and be excreted in the urine.

Absorption, distribution, metabolism and elimination

The menthyl esters in this group (Nos 1852, 1854, 1855 and 1858) can be expected to be readily hydrolysed to menthol and their respective carboxylic acids. Carboxylesterases are found in the endoplasmic reticulum of most mammalian tissues, but occur predominantly in hepatocytes. The metabolites of menthol are eliminated in the urine and/or faeces either unchanged or conjugated with glucuronic acid. The ketal (No. 1859) is expected to be hydrolysed to yield (−)- or (±)-menthone and simple glycols. The ketone, menthone, is primarily reduced to the corresponding secondary alcohol, neo-menthol, which is metabolized and eliminated by pathways similar to those of its stereoisomer, menthol. The ketones (Nos 1856 and 1857) in this group would be reduced to their corresponding secondary alcohols and conjugated mainly with glucuronic acid. The alicyclic alcohols (Nos 1853, 1860 and 1861) are expected to be conjugated mainly with glucuronic acid and eliminated in the urine or faeces.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to this group of flavouring agents, the Committee assigned five of the flavouring agents (Nos 1852–1855 and 1860) to structural class I. The remaining five flavouring agents (Nos 1856–1859 and 1861) were assigned to structural class II.

Step 2. All the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily per capita intakes of all five of the flavouring agents in structural class I are below the threshold of concern (i.e. 1800 μg/person per day for class I). The estimated daily per capita intakes of all five of the flavouring agents in structural class II are below the threshold of concern (i.e. 540 μg/person per day for class II).

Table 16 summarizes the evaluations of the 10 additional flavouring agents structurally related to menthol (Nos 1852–1861) in this group.

Consideration of combined intakes from use as flavouring agents

The daily intakes of the 10 additional flavouring agents structurally related to menthol considered in this group are all relatively low in comparison with those of the previously considered flavouring agents in this group (Annex 1, reference *137*). In the unlikely event that the flavouring agents in structural class I with the common metabolite menthol (No. 427) from this group

(Nos 1852, 1854, 1855 and 1858) and the previously evaluated group (Nos 432, 433 and 447) were to be consumed together with menthol (No. 427) on a daily basis, the estimated combined intakes[1] would be 1527 μg/person per day in Europe and 3513 μg/person per day in the USA. The estimated combined intake would therefore exceed the human threshold of concern (i.e. 1800 μg/person per day for class I) in the USA. However, the vast majority of the combined intake would be due to menthol per se, which has an ADI of 0–4 mg/kg bw established by the Committee at its fifty-first meeting (Annex 1, reference *37*). Also, all 10 flavouring agents and the 14 flavouring agents considered previously are expected to be metabolized efficiently and would not saturate available metabolic pathways. The overall evaluation of the data indicated that combined intake would not raise safety concerns.

Consideration of secondary components

No flavouring agents in this group have minimum assay values of less than 95%.

Conclusion

In the previous evaluations of substances in this group, studies of acute toxicity, short-term and long-term studies of toxicity and studies of genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation supported those from the previous evaluations.

The Committee concluded that these 10 flavouring agents, which are additions to the group of substances structurally related to menthol evaluated previously, do not give rise to safety concerns at the current estimated levels of intake.

An addendum to the monograph (Annex 1, reference *137*) summarizing the safety data on this group of flavouring agents was prepared.

4.2 Re-evaluation of flavouring agents for which estimated intake was based on anticipated poundage data

The Procedure for the Safety Evaluation of Flavouring Agents employs a decision tree approach for the safety evaluation of flavouring agents, using a TTC based on structural class and a measure of estimated daily intake for each substance. For the estimated daily intake, the Procedure uses the MSDI

[1] Combined intake was calculated on a molar basis relative to the formation of a common metabolite. In this case, the common metabolite is menthol, with a relative molecular mass of 156.69.

(also known as "per capita times 10") for a substance, which is derived from a reported annual volume of production.

The annual volumes of production submitted to the Committee by the flavour industry were gathered from periodic surveys of flavour manufacturers. At the fifty-ninth, sixty-first, sixty-third and sixty-fifth meetings of the Committee (Annex 1, references *160*, *166*, *173* and *178*), only "anticipated" annual volumes of productions were provided for some flavouring agents and used in the MSDI calculation. These volumes were used for expedience in completing a safety evaluation, but the conclusions of the Committee were made conditional pending the submission of actual poundage data.

Actual production volumes were subsequently submitted for all 143 requested flavouring agents and were evaluated by the Committee at its present meeting (see Table 17). Two of these substances (Nos 1438 and 1439) were included erroneously in the call for data; these are natural L-amino acids, and the Committee had previously concluded that it is not appropriate to evaluate these compounds via the Procedure, since they are natural components of the diet in amounts that are orders of magnitude higher than the anticipated levels of exposure from use as flavouring agents (Annex 1, reference *173*).

Table 17

Summary of re-evaluation of flavouring agents for which estimated intake was based on anticipated poundage data when previously evaluated at the fifty-ninth, sixty-first, sixty-third and sixty-fifth meetings

JECFA No.	Flavouring substance	Structural class[a]	Anticipated[b] poundage (kg)	USA 2008 Reported[c] annual volume (kg)	USA intake[d] (µg/day)	USA intake[d] (µg/kg bw per day)	Europe 2005 Reported[c] annual volume (kg)	Europe intake[d] (µg/day)	Europe intake[d] (µg/kg bw per day)	Japan 2005 Reported[c] annual volume (kg)	Japan intake[d] (µg/day)	Japan intake[d] (µg/kg bw per day)	Assessment[e]	Conclusion
963	Ethyl cyclohexanecarboxylate	I	0.91	1	0.1	0.002	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
986	10-Hydroxymethylene-2-pinene	I	0.1	127	16	0.3	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1063	2,5-Dimethyl-3-furanthiol	II	4	1	0.1	0.002	0.3	0.03	0.0005	0.1	0.03	0.0004	A	No safety concern
1065	Propyl 2-methyl-3-furyl disulfide	II	4	0.5	0.06	0.001	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1066	Bis(2-methyl-3-furyl) disulfide	II	4	0.1	0.01	0.0002	20	2	0.04	1	0.3	0.006	A	No safety concern
1067	Bis(2,5-dimethyl-3-furyl) disulfide	III	4	2	0.2	0.004	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1068	Bis(2-methyl-3-furyl) tetrasulfide	III	4	4	0.5	0.008	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern
1070	2,5-Dimethyl-3-furan thioisovalerate	III	4	2	0.2	0.004	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1077	Furfuryl isopropyl sulfide	III	1	0	0.01	0.0002	ND	ND	ND	ND	ND	ND	A	No safety concern
1082	2-Methyl-3, 5- or 6-(furfurylthio)pyrazine	III	4	0.7	0.09	0.001	0.1	0.01	0.0002	1	0.4	0.006	A	No safety concern
1085	3-[(2-Methyl-3-furyl)thio]-4-heptanone	III	4	11	1	0.02	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern

1086	2,6-Dimethyl-3-[(2-methyl-3-furyl)thio]-4-heptanone	III	4	7	0.9	0.01	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern
1087	4-[(2-Methyl-3-furyl)thio]-5-nonanone	III	4	3	0.4	0.006	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1089	2-Methyl-3-thioacetoxy-4,5-dihydrofuran	III	4	2	0.2	0.004	69	7	0.1	ND	ND	ND	B	No safety concern
1157	4-Hydroxy-4-methyl-5-hexenoic acid gamma-lactone	I	18	14	2	0.03	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1158	(±) 3-Methyl-gamma-decalactone	I	30	37	5	0.08	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1159	4-Hydroxy-4-methyl-7-*cis*-decenoic acid gamma-lactone	I	75	829	101	2	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern
1160	Tuberose lactone	I	60	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1161	Dihydromintlactone	III	66	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1162	Mintlactone	III	50	11	1	0.02	72	8	0.1	0.9	0.2	0.004	B	No safety concern
1163	Dehydromenthofurolactone	III	50	0.1	0.01	0.0002	10	1	0.02	8	2	0.03	A	No safety concern
1164	(±)-(2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid gamma-lactone	III	5	0.4	0.05	0.0008	169	18	0.3	13	3	0.06	B	No safety concern
1167	2-(4-Methyl-2-hydroxyphenyl)propionic acid gamma-lactone	III	9	ND	ND	ND	7	0.7	0.01	ND	ND	ND	A	No safety concern
1174	2,4-Hexadien-1-ol	I	2	10	1	0.02	0.1	0.01	0.0002	0.4	0.1	0.002	B	No safety concern
1176	(*E*,*E*)-2,4-Hexadienoic acid[f]	I	37	229	28	0.5	134	14	0.2	0.1	0.03	0.0004	B	No safety concern
1180	(*E*,*E*)-2,4-Octadien-1-ol	I	100	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern

1183	2,4-Nonadien-1-ol	I	150	3	0.4	0.006	0.5	0.05	0.0009	0.1	0.04	0.0006	A	No safety concern
1188	(*E*,*Z*)-2,6-Nonadien-1-ol acetate[f]	I	100	3	0.4	0.006	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1189	(*E*,*E*)-2,4-Decadien-1-ol	I	150	21	3	0.04	7466	799	13	ND	ND	ND	B	No safety concern
1191	Methyl (*E*)-2-(*Z*)-4-decadienoate	I	4.5	0.4	0.05	0.0008	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1193	Ethyl 2,4,7-decatrienoate[f]	I	2.4	0.1	0.01	0.0002	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1199	(±) 2-Methyl-1-butanol	I	200	96	12	0.2	1103	118	2	ND	ND	ND	B	No safety concern
1217	2-Methyl-2-octenal	I	45	7	0.9	0.01	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1218	4-Ethyloctanoic acid	I	23	0.1	0.01	0.0002	6	0.6	0.01	0.1	0.03	0.0004	A	No safety concern
1226	8-Ocimenyl acetate	I	44	31	4	0.06	20	2	0.04	ND	ND	ND	A	No safety concern
1228	3,7,11-Trimethyl-2,6,10-dodecatrienal	I	1.3	ND	ND	ND	4	0.4	0.007	ND	ND	ND	B	No safety concern
1229	12-Methyltridecanal	I	3	5	0.6	0.01	0.5	0.05	0.0009	0.1	0.03	0.0004	B	No safety concern
1232	1-Ethoxy-3-methyl-2-butene	II	11	0.1	0.01	0.0002	176	19	0.3	2	0.5	0.008	B	No safety concern
1236	2,2,6-Trimethyl-6-vinyltetrahydropyran	II	45	1	0.1	0.002	0.2	0.02	0.0004	56	15	0.2	B	No safety concern
1239	Cycloionone	II	9	ND	ND	ND	1	0.1	0.002	ND	ND	ND	A	No safety concern
1245	2,4-Dimethylanisole[f]	I	1	3	0.4	0.006	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern
1248	1,2-Dimethoxybenzene	I	113	ND	ND	ND	13	1	0.02	0.3	0.08	0.001	A	No safety concern
1265	4-Propenyl-2,6-dimethoxyphenol	I	11	6	0.7	0.01	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1289	erythro- and threo-3-Mercapto-2-methylbutan-1-ol	I	10	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern

1290	(±)-2-Mercapto-2-methylpentan-1-ol	I	20	25	3	0.05	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1292	3-Mercapto-2-methylpentanal	I	20	ND	ND	ND	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1293	4-Mercapto-4-methyl-2-pentanone	I	0.1	0.3	0.04	0.0006	5	0.5	0.009	1	0.3	0.004	B	No safety concern
1296	spiro[2,4-Dithia-1-methyl-8-oxabicyclo(3.3.0) octane-3,3'-(1'-oxa-2'-methyl)-cyclopentane]	III	14	632	77	1	50	5	0.09	5	1	0.02	B	No safety concern
1299	2,3,5-Trithiahexane	I	0.2	ND	ND	ND	0.3	0.03	0.0005	0.1	0.03	0.0004	B	No safety concern
1300	Diisopropyl trisulfide	I	0.04	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1311	2-(2-Methylpropyl)pyridine	III	5	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1319	2-Propionylpyrrole	II	11	ND	ND	ND	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1322	2-Propylpyridine	II	5	4	0.5	0.008	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1334	4-Methylbiphenyl	III	0.5	1	0.1	0.002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1342	*d*-3-Carene	I	225	115	14	0.2	2351	252	4	0.9	0.2	0.004	B	No safety concern
1343	Farnesene (alpha and beta)	I	225	2	0.2	0.004	5	0.5	0.009	2	0.5	0.008	A	No safety concern
1344	1-Methyl-1,3-cyclohexadiene	I	1783	2	0.2	0.004	ND	ND	ND	ND	ND	ND	A	No safety concern
1367	*trans*-2-Octen-1-yl acetate	I	4	13	2	0.03	0.4	0.04	0.0007	0.1	0.03	0.0004	B	No safety concern
1368	*trans*-2-Octen-1-yl butanoate	I	4	2	0.2	0.004	2	0.2	0.004	0.3	0.07	0.001	A	No safety concern
1369	*cis*-2-Nonen-1-ol	I	11	3	0.4	0.006	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1370	(*E*)-2-Octen-1-ol	I	1	0.4	0.05	0.0008	13	1	0.02	0.1	0.03	0.0006	B	No safety concern

1371	(*E*)-2-Butenoic acid	I	40	ND	ND	ND	0.3	0.03	0.0005	40	10	0.2	B	No safety concern
1372	(*E*)-2-Decenoic acid	I	25	0.1	0.01	0.0002	0.1	0.01	0.0002	18	5	0.08	A	No safety concern
1373	(*E*)-2-Heptenoic acid	I	20	18	2	0.04	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1374	(*Z*)-2-Hexen-1-ol	I	55	17	2	0.03	5	0.5	0.009	0.1	0.03	0.0004	A	No safety concern
1375	*trans*-2-Hexenyl butyrate	I	21	0.1	0.01	0.0002	10	1	0.02	60	16	0.3	B	No safety concern
1376	(*E*)-2-Hexenyl formate	I	40	0.3	0.04	0.0006	0.6	0.06	0.001	0.5	0.1	0.002	A	No safety concern
1377	*trans*-2-Hexenyl isovalerate	I	20	0.1	0.01	0.0002	130	14	0.2	0.1	0.03	0.0004	B	No safety concern
1378	*trans*-2-Hexenyl propionate	I	22	151	18	0.3	0.5	0.05	0.0009	1	0.4	0.006	B	No safety concern
1379	*trans*-2-Hexenyl pentanoate	I	25	1	0.1	0.002	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1380	(*E*)-2-Nonenoic acid	I	15	0.1	0.01	0.0002	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1381	(*E*)-2-Hexenyl hexanoate	I	0.5	1	0.1	0.002	1	0.1	0.002	12	3	0.05	B	No safety concern
1382	(*Z*)-3- and (*E*)-2-Hexenyl propionate	I	5	30	4	0.06	ND	ND	ND	ND	ND	ND	B	No safety concern
1384	2-Undecen-1-ol	I	3	0.3	0.04	0.0006	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1407	Dihydronootkatone	II	5	0.3	0.04	0.0006	2	0.2	0.004	25	7	0.1	B	No safety concern
1409	beta-Ionyl acetate	I	50	55	7	0.1	23	2	0.04	ND	ND	ND	B	No safety concern
1410	alpha-Isomethylionyl acetate	I	50	5	0.6	0.01	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1411	3-(*l*-Menthoxy)-2-methylpropane-1,2-diol	I	500	100	12	0.2	0.1	0.01	0.0002	7	2	0.03	A	No safety concern
1412	Bornyl butyrate[f]	I	50	17	2	0.03	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern

1413	*d,l*-Menthol-(±)-propylene glycol carbonate	I	800	3626	443	7	315	34	0.6	ND	ND	ND	B	No safety concern
1414	**L-Monomenthyl glutarate[g]**	**I**	**750**	**46 465**	**5683**	**95**	**4090**	**438**	**7**	**ND**	**ND**	**ND**	**C**	**Re-evaluation**
1415	L-Menthyl methyl ether	III	300	1	0.1	0.002	ND	ND	ND	ND	ND	ND	A	No safety concern
1416	*p*-Menthane-3,8-diol	I	100	7	0.9	0.01	0.1	0.01	0.0002	322	85	1	B	No safety concern
1435	Taurine	I	8250	2149	263	4	6317	676	11	ND	ND	ND	A	No safety concern
1438	L-Arginine	I	8600	4657	570	9	8266	885	15	ND	ND	ND		Not appropriate to evaluate via Procedure, natural component of the diet at much higher amounts.
1439	L-Lysine	I	8600	7146	874	15	4183	448	7	ND	ND	ND		See above
1447	Tetrahydrofurfuryl cinnamate[f]	III	0.05	0.1	0.01	0.0002	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern
1457	(±)-2-(5-Methyl-5-vinyltetrahydrofuran-2-yl) propionaldehyde	II	5	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1475	Ethyl 2-ethyl-3-phenylpropanoate	I	5	0.5	0.06	0.001	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1478/1479	2-Oxo-3-phenylpropionic acid and 2-oxo-3-phenylpropionic acid sodium salt	I	0.5	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1483	2-Methyl-3-(1-oxopropoxy)-4H-pyran-4-one[f]	I	150	243	30	0.5	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern

1527	4-Allylphenol	I	0.6	0.5	0.06	0.001	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1528	2-Methoxy-6-(2-propenyl) phenol	I	1	0.1	0.01	0.0002	ND	ND	ND	ND	ND	ND	A	No safety concern
1532	Eugenyl isovalerate	I	3	3	0.4	0.006	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1538	*cis*-3-Hexenyl anthranilate	I	300	81	10	0.2	0.1	0.01	0.0002	86	23	0.4	A	No safety concern
1539	Citronellyl anthranilate	I	50	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1546	Ethyl *N*-methylanthranilate	I	0.2	0.5	0.06	0.001	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1547	Ethyl *N*-ethylanthranilate	I	0.5	0.2	0.02	0.0004	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1548	Isobutyl *N*-methylanthranilate	I	0.5	ND	ND	ND	4	0.4	0.007	0.1	0.03	0.0004	B	No safety concern
1549	Methyl *N*-formylanthranilate	I	1	ND	ND	ND	0.1	0.01	0.0002	1	0.3	0.004	B	No safety concern
1550	Methyl *N*-acetylanthranilate	I	0.3	ND	ND	ND	16	2	0.03	8	2	0.04	B	No safety concern
1551	Methyl *N*,*N*-dimethylanthranilate	I	102	11	1	0.02	0.1	0.01	0.0002	4	1	0.02	A	No safety concern
1552	*N*-Benzoylanthranilic acid	I	10	9	1	0.02	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1553	Trimethyloxazole	II	8	ND	ND	ND	16	2	0.03	1	0.3	0.004	B	No safety concern
1554	2,5-Dimethyl-4-ethyloxazole	II	1.3	0.1	0.01	0.0002	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1555	2-Ethyl-4,5-dimethyloxazole	II	4	ND	ND	ND	0.1	0.01	0.0002	0.1	0.03	0.0004	A	No safety concern
1556	2-Isobutyl-4,5-dimethyloxazole	II	1.3	0.9	0.1	0.002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1557	2-Methyl-4,5-benzo-oxazole	II	0.7	0.2	0.02	0.0004	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1558	2,4-Dimethyl-3-oxazoline	II	0.5	0.2	0.02	0.0004	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern

1561	Butyl isothiocyanate	II	11	ND	ND	ND	0.4	0.04	0.0007	4	1	0.02	A	No safety concern
1562	Benzyl isothiocyanate	II	2.5	ND	ND	ND	9	1.0	0.02	0.2	0.05	0.0009	B	No safety concern
1563	Phenethyl isothiocyanate	II	2.8	0.1	0.01	0.0002	175	19	0.3	56	15	0.2	B	No safety concern
1569	4,5-Dimethyl-2-propyloxazolef	II	0.8	5	0.6	0.01	0.1	0.01	0.0002	0.10	0.03	0.0004	B	No safety concern
1570	4,5-Epoxy-(*E*)-2-decenal	III	1	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1571	beta-Ionone epoxide	III	0.6	ND	ND	ND	1	0.1	0.002	0.1	0.03	0.0004	B	No safety concern
1573	Epoxyoxophorone	III	1	0.8	0.10	0.002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1579	Ethylamine	I	1	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1580	Propylamine	I	0.1	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1581	Isopropylamine	I	0.1	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1583	Isobutylamine	I	0.5	0.6	0.07	0.001	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1584	*sec*-Butylamine	I	14	14	2	0.03	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1585	Pentylamine	I	1	ND	ND	ND	0.3	0.03	0.0005	ND	ND	ND	A	No safety concern
1586	2-Methylbutylamine	I	0.1	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1588	Hexylamine	I	0.04	ND	ND	ND	0.2	0.02	0.0004	ND	ND	ND	B	No safety concern
1590	2-(4-Hydroxyphenyl) ethylamine	II	0.1	10	1	0.02	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1591	1-Amino-2-propanol	I	90	ND	ND	ND	15	2	0.03	ND	ND	ND	A	No safety concern
1593	Butyramide	II	0.01	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern

1594	1,6-Hexalactam	III	0.01	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1595	**2-Isopropyl-*N*,2,3-trimethylbutyramide[g]**	**III**	**6000**	**54 066**	**6613**	**110**	**193**	**21**	**0.3**	**ND**	**ND**	**ND**	**C**	**Re-evaluation**
1596	*N*-Ethyl (*E*)-2,(*Z*)-6-nonadienamide	III	500	2	0.2	0.004	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1597	*N*-Cyclopropyl (*E*)-2,(*Z*)-6-nonadienamide	III	225	2	0.2	0.004	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1598	*N*-Isobutyl (*E*,*E*)-2,4-decadienamide	III	470	2	0.2	0.004	18	2	0.03	ND	ND	ND	A	No safety concern
1602	(±)-*N*,*N*-Dimethyl menthyl succinamide	I	500	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1603	1-Pyrroline	II	2	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1604	2-Acetyl-1-pyrroline	II	0.6	0.9	0.1	0.002	0.1	0.01	0.0002	0.1	0.03	0.0004	B	No safety concern
1605	2-Propionylpyrroline	II	1	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	A	No safety concern
1606	Isopentylidene isopentylamine	III	0.06	ND	ND	ND	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1608	2-Methylpiperidine	II	0.01	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1611	Triethylamine	I	5	5	0.6	0.01	0.6	0.06	0.001	ND	ND	ND	B	No safety concern
1612	Tripropylamine	I	0.1	0.1	0.01	0.0002	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1613	*N*,*N*-Dimethylphenethylamine	III	0.5	0.5	0.06	0.001	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern
1614	Trimethylamine oxide	I	0.5	0.6	0.07	0.001	19	2	0.03	ND	ND	ND	B	No safety concern
1615	Piperazine	II	0.01	0.7	0.09	0.001	0.1	0.01	0.0002	ND	ND	ND	B	No safety concern

ND, no intake data reported.

[a] Reference *2*.

[b] The volume cited is the anticipated annual volume, which was the maximum amount of flavour estimated to be used annually by the manufacturer at the time the material was proposed for flavour use.

[c] From references *14*, *15* and *16*. Total poundage values of <0.1 kg reported in the surveys (*14*, *15*, *16*) have been truncated to one place following the decimal point (0.1 kg).

[d] Intake (μg/person per day) calculated as follows: [(annual volume, kg) × (1 × 109 μg/kg)/(population × survey correction factor × 365 days)], where population (10%, "consumers only") = 32×10^6 for Europe, 28×10^6 for the USA and 13×10^6 for Japan; and where survey correction factor = 0.8 for surveys by the USA, Europe and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*14*, *15*, *16*).
The consumption ratio is calculated as follows: (annual consumption from food, kg)/(most recent reported volume as a flavouring substance, kg).

[e] For assessment:
A actual poundage < anticipated poundage; MSDI < TTC
B actual poundage ≥ anticipated poundage; MSDI < TTC
C actual poundage > anticipated poundage; MSDI > TTC

[f] Reference *16*.

[g] *Step B3*: According to the Procedure (Annex 1, reference *131*), more extensive data on metabolism and toxicity must be considered to complete the safety evaluation of L-monomenthyl glutarate (No. 1414) and 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595), for which intake levels estimated from the use of these compounds as flavouring agents were determined to exceed the threshold of concern for structural class I (i.e. 1800 μg/person per day) and structural class III (i.e. 90 μg/person per day), respectively.

Of the other 141 compounds, 85 were in structural class I; for 43 of these, the actual volumes of production were lower than the anticipated volumes, while for 42 substances, the actual production volumes were higher than the anticipated ones. For only one of these substances (No. 1414) did the higher actual production volume lead to an intake estimate that exceeded the TTC of 1800 μg/person per day for the class, hence requiring a re-evaluation.

There were 27 substances in structural class II; 15 had actual volumes of production that were lower than those previously anticipated, and 12 had actual production volumes that were higher than those previously anticipated, but the intake estimates did not exceed the class threshold.

There were 29 substances in structural class III; 16 of these had actual volumes of production that were lower than those previously anticipated, while 13 had actual production volumes that were higher than those previously anticipated. For only one of these substances (No 1595) did the higher actual production volume lead to an intake estimate that exceeded the TTC of 90 μg/person per day for the class, hence requiring a re-evaluation.

The Committee reiterated its position stated at the sixty-fifth meeting, which "emphasized that flavouring agents should be evaluated on the basis of complete, up-to-date information".

The two flavouring substances requiring a re-evaluation were 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595) and L-monomenthyl glutarate (No. 1414).

4.2.1 *2-Isopropyl-N,2,3-trimethylbutyramide (No. 1595)*

2-Isopropyl-*N*,2,3-trimethylbutyramide (No. 1595) was previously evaluated by the Committee at its sixty-fifth meeting together with 37 other flavouring agents belonging to the group of aliphatic and aromatic amines and amides (Annex 1, reference *178*).

Toxicological data

For the previous evaluation, studies of genotoxicity with 2-isopropyl-*N*, 2,3-trimethylbutyramide in vitro were available to the Committee, as well as several studies of toxicity in rats treated with 2-isopropyl-*N*,2,3-trimethylbutyramide by gavage (Annex 1, references *178*, *179*). In these studies, 2-isopropyl-*N*,2,3-trimethylbutyramide showed no genotoxic potential, was of moderate acute toxicity and induced no reproductive or teratogenic effects. The short-term studies of toxicity showed treatment-related hepatic and renal toxicity at doses of 10 mg/kg bw and greater. The Committee at its previous

meeting identified a NOEL[1] of 5 mg/kg bw per day for 2-isopropyl-*N*,2,3-trimethylbutyramide on the basis of histopathological lesions in the kidneys of male rats in a 14-week study.

Additional studies of toxicity with 2-isopropyl-*N*,2,3-trimethylbutyramide were available to the Committee at its present meeting. These studies confirmed the moderate acute toxicity of orally administered 2-isopropyl-*N*,2,3-trimethylbutyramide and the absence of mutagenic potential in bacteria in vitro. However, in mammalian cells in vitro, 2-isopropyl-*N*,2,3-trimethylbutyramide showed evidence of clastogenicity in the presence, but not in the absence, of metabolic activation. Furthermore, in a 90-day dietary study in rats, treatment with 2-isopropyl-*N*,2,3-trimethylbutyramide was associated with hepatic effects with a NOAEL of 25 mg/kg bw per day; however, no renal effects were observed.

The Committee expressed its concern that the observed clastogenicity might be due to formation of a reactive metabolite, but the mechanism has not been studied. The Committee also noted the inconsistencies in the effects observed on the kidneys: renal effects were seen in two 14-week gavage studies but not in a 90-day dietary study in which 2-isopropyl-*N*,2,3-trimethylbutyramide was given at comparable or slightly higher doses. Moreover, the kidney effects observed differed in the two 14-week gavage studies: renal tubular nephrosis was found in male and female rats in one study, while tubular dilatation with granular casts and hyaline droplet formation was found in male rats only in the other study. Accordingly, the Committee expressed concern as to how to address these effects and identify a NOAEL.

Conclusion

The Committee concluded that the Procedure could not be applied to 2-isopropyl-*N*,2,3-trimethylbutyramide because of the above concerns. Information that would assist in resolving the concerns would include data on the potential of this compound to form reactive metabolites and on whether clastogenicity is also expressed in vivo, as well as additional information on the kidney effects found at relatively low doses.

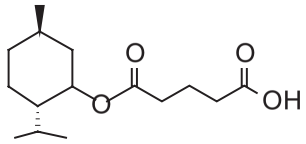
4.2.2 *L-Monomenthyl glutarate (No. 1414)*

L-Monomenthyl glutarate (No. 1414; see Table 18) was previously evaluated by the Committee at its sixty-third meeting together with 31 other flavouring agents belonging to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters (Annex 1, reference *173*). The Committee re-evaluated L-monomenthyl glutarate by the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*).

[1] According to the decision taken by the Committee at its sixty-eighth meeting (Annex 1, reference *187*), this would now be termed a NOAEL.

Table 18

Summary of results of the safety re-evaluation of the flavouring agent L-monomenthyl glutarate (No. 1414)[a,b]

Flavouring agent	No.	CAS No. and structure	*Step A3*[c] Does the estimated intake exceed the threshold for human intake?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the agent or related substance?	Comments on predicted metabolism	Conclusion based on current estimated intake
Structural class I							
L-Monomenthyl glutarate	1414	220621-22-7 	Yes Europe: 438 USA: 5683 Japan: ND	No	Yes. The NOEL[d] of 380 mg/kg bw per day (*32*) for the hydrolysis product menthol (No. 427) is at least 4000 times the estimated daily intake of L-monomenthyl glutarate when used as a flavouring agent.	See notes 1 and 2	No safety concern

CAS, Chemical Abstracts Service; ND, no intake data reported.

[a] L-Monomenthyl glutarate (No. 1414) was previously evaluated by the Committee at its sixty-third meeting, along with 31 other flavouring agents belonging to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters (Annex 1, reference *173*).

[b] *Step 2:* L-Monomenthyl glutarate is expected to be metabolized to innocuous products.

[c] The threshold for human intake for structural class I is 1800 µg/person per day. All intake values are expressed in µg/day.

[d] According to the decision taken by the Committee at its sixty-eight meeting (Annex 1, reference 187), this would not be termed a NOAEL.

Notes:
1. Ester hydrolysis to liberate the corresponding alcohol (menthol) and carboxylic acid (glutaric acid).
2. Formation of glucuronic acid conjugates directly or after metabolism, which are subsequently excreted in the urine.

L-Monomenthyl glutarate is structurally related to menthol. Menthol was previously evaluated by the Committee at its eleventh, eighteenth, twentieth and fifty-first meetings (Annex 1, references *14*, *35*, *41* and *137*), and was allocated an ADI of 0–4 mg/kg bw at the fifty-first meeting.

The Committee previously evaluated a group of 13 flavouring agents structurally related to menthol at its fifty-first meeting (Annex 1, reference *137*). It was then concluded that neither the 13 substances in that group nor menthol was of safety concern at the current estimated levels of intake. The findings from these evaluations were also considered in the present re-evaluation.

Assessment of dietary exposure

Recent surveys undertaken by the flavour industry associations in Europe, Japan and the USA revealed use of L-monomenthyl glutarate as a flavouring agent in Europe and the USA. Annual volumes of production for L-monomenthyl glutarate are reported in Table 19 and are approximately 4090 kg in Europe (*14*) and 46 465 kg in the USA (*15*). The daily per capita intakes of L-monomenthyl glutarate in Europe and the USA are reported in Table 18 and are 438 and 5683 µg/person, respectively.

Table 19

Annual volumes of production of L-monomenthyl glutarate (No. 1414) when used as a flavouring agent in Europe, the USA and Japan[a]

Flavouring agent (No.)	Reported[b] annual volume (kg)	Intake[c]	
		µg/day	µg/kg bw per day
L-Monomenthyl glutarate (1414)			
Europe	4090	438	7
USA	46465	5683	95
Japan	ND	ND	ND

ND, no intake data reported.

[a] L-Monomenthyl glutarate (No. 1414) was previously evaluated by the Committee at its sixty-third meeting, along with 31 other flavouring agents belonging to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters (Annex 1, reference *173*).

[b] From references *14*, *15* and *16*.

[c] Intake (µg/person per day) calculated as follows: [(annual volume, kg) × (1 x 10^9 µg/kg)/(population × survey correction factor × 365 days)], where population (10%, "consumers only") = 32 × 10^6 for Europe, 28 × 10^6 for the USA and 13 × 10^6 for Japan; and where survey correction factor = 0.8 for surveys by the USA, Europe and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*14*, *15*, *16*).
Intake (µg/kg bw per day) calculated as follows: (µg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur from rounding.

Absorption, distribution, metabolism and elimination

No relevant additional data on the absorption, distribution, metabolism and elimination of L-monomenthyl glutarate or structurally related substances, including menthol, have been reported since the previous evaluations (Annex 1, references *137* and *173*). After absorption, L-monomenthyl glutarate, like other menthyl esters, is expected to be readily hydrolysed in humans to menthol and its respective carboxylic acid (i.e. glutaric acid). The latter is endogenous in humans and metabolized via decarboxylation and β-oxidation. Menthol is not endogenous in humans. It is efficiently metabolized by a combination of oxidation and, mainly, conjugation with glucuronic acid, resulting in innocuous products. Elimination is largely as glucuronides, mostly in the urine.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned L-monomenthyl glutarate (No. 1414) to structural class I (*2*).

Step 2. L-Monomenthyl glutarate (No. 1414) is expected to be metabolized to innocuous products. The evaluation of this flavouring agent therefore proceeded via the A-side of the Procedure.

Step A3. The estimated daily intake of L-monomenthyl glutarate (No. 1414), which is 438 μg/person in Europe and 5683 μg/person in the USA, exceeds the threshold of concern (i.e. 1800 μg/person per day for class I). Accordingly, the evaluation of L-monomenthyl glutarate proceeded to step A4.

Step A4. L-Monomenthyl glutarate and one of its hydrolysis products (menthol, No. 427) are not endogenous in humans. Therefore, its evaluation proceeded to step A5.

Step A5. The NOEL of 380 mg/kg bw per day for the hydrolysis product menthol (No. 427) from a 2-year study of carcinogenicity in rats (*32*) is at least 4000 times greater than the estimated intake of the parent compound L-monomenthyl glutarate (No. 1414) from its use as a flavouring agent in Europe (7 μg/kg bw per day) and the USA (95 μg/kg bw per day). The Committee therefore concluded that L-monomenthyl glutarate was of no safety concern at the current estimated levels of intake.

Table 18 summarizes the re-evaluation of L-monomenthyl glutarate (No. 1414).

Toxicological data

Since the previous evaluations, no additional toxicological studies have become available on L-monomenthyl glutarate or structurally related substances, including menthol. It was concluded that these substances are of low acute toxicity when administered orally and that there is no evidence of genotoxic or teratogenic potential (Annex 1, references *137* and *173*). No short-term or long-term studies of toxicity were available for L-monomenthyl glutarate. For its hydrolysis product menthol, however, an ADI of 0–4 mg/kg bw was established on the basis of a NOEL of 380 mg/kg bw per day, the highest dose tested in a 2-year study of carcinogenicity in rats (Annex 1, reference *137*).

Consideration of combined intakes from use as flavouring agents

In the unlikely event that L-monomenthyl glutarate is consumed together with menthol (No. 427, structural class I) and other menthol derivatives concurrently on a daily basis, the combined intakes for the five flavouring agents with the highest intakes (Nos 427, 1414, 429, 443 and 431) would be 19 597 µg and 16 745 µg in Europe and the USA, respectively, and would exceed the threshold of concern for class I (i.e. 1800 µg/person per day). However, these five agents are all expected to be metabolized efficiently. Moreover, the combined intakes do not exceed the ADI of 0–4 mg/kg bw for menthol, as established by the Committee at its fifty-first meeting (Annex 1, reference *137*). The Committee concluded that under the conditions of use as flavouring agents, the combined intake of substances leading to the common metabolite menthol would not saturate the metabolic pathways and the combined intake does not raise safety concerns.

Consideration of secondary components

According to the previous evaluation (Annex 1, reference *173*), L-monomenthyl glutarate has a minimum assay value of less than 95%. The secondary components are dimenthyl glutarate (22–24%) and glutaric acid (1–2%). Both secondary components were concluded not to present a safety concern on the basis of the following considerations. Glutaric acid is endogenous and of low toxicity. Dimenthyl glutarate is expected to follow the same metabolic pathway as L-monomenthyl glutarate — i.e. ester hydrolysis yielding menthol and glutaric acid. Menthol (No. 427) has previously been evaluated by the Committee and was concluded to be of no safety concern (Annex 1, reference *137*).

Conclusion

The Committee concluded that the flavouring agent L-monomenthyl glutarate (No. 1414), which was previously evaluated along with 31 other flavouring agents belonging to the group of monocyclic and bicyclic secondary alcohols, ketones and related esters (Annex 1, reference *173*), does not give rise to safety concerns at the current estimated levels of intake.

No new toxicological monograph was prepared.

4.3 Specifications of identify and purity of flavouring agents

Specifications monographs were prepared for the 111 flavouring agents placed on the agenda for the first time at the present meeting. The Committee confirmed the importance of correct structural formulae, including information on geometric/optical isomers, for the preparation of specifications on identity and purity and for the safety evaluation.

The specifications prepared for the group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers by the Committee at its sixty-fifth and sixty-eighth meetings (Annex 1, references *178* and *187*) and the specifications prepared for the six alkoxy-substituted allylbenzenes by the Committee at its present meeting will include a statement that the safety evaluations for these substances had not been completed.

5. Future work

Considerations on the thresholds of toxicological concern used in the Procedure for the Safety Evaluation of Flavouring Agents

The Committee is aware that there are various activities currently under way to update and revise the Cramer decision tree (*2*), which is used to determine the structural class, and also to update the toxicology database used to establish the TTC values. There is widespread interest in developing TTC values appropriate to specific applications, such as flavouring agents, certain food additives and residues of pesticides and veterinary drugs in food. The Committee considered that this subject should be discussed in depth at a future meeting.

Incorporation of the SPET estimate into the Procedure for the Safety Evaluation of Flavouring Agents

The Committee concluded that it was necessary to incorporate SPET estimates into the Procedure for all flavouring agents considered at future meetings of the Committee. The Committee agreed that it would not be necessary to re-evaluate flavouring agents that have already been assessed using the Procedure.

Mineral oils (low and medium viscosity), classes II and III

The re-evaluation of the safety of mineral oils (low and medium viscosity), classes II and III, was deferred to a future meeting. The Committee received information from the sponsor that relevant studies are being undertaken and agreed to maintain the temporary ADI until the end of 2009, awaiting additional data to be submitted.

Paprika extract

The Committee recommended that the specifications for paprika oleoresin be revised at a future meeting in order to allow the differentiation of paprika extract used as a colour from paprika oleoresin used as a flavour.

6. Recommendations

Incorporation of the SPET estimate into the Procedure for the Safety Evaluation of Flavouring Agents

To enable a safety evaluation using the Procedure to be undertaken, the Committee requested that added use level data be provided for each flavouring agent in a timely fashion before the meeting, in addition to up-to-date data on production volumes, as part of the data package for the safety evaluation. The Committee will not perform a safety evaluation in the absence of such data.

Relationship between the ADI and specifications

The Committee recommends that when proposals are made to include or revise limits for impurities or when compositional changes occur that lead to a need for revision of the specifications, the consequences for the safety assessment of the substance need to be considered.

Considerations on potentially necessary data requirements and re-evaluation of the safety of the specified material need to be taken into account by the JECFA Secretariat and by CCFA when requesting changes to existing specifications.

Sulfites: dietary exposure assessment and maximum levels (MLs) in foods

Countries that have not yet done so could consider collecting data on the current use of sulfites in food and beverages available on their markets and investigating whether dietary exposure in some subpopulations exceeds the ADI. On the basis of this investigation, individual countries and the food industry could consider the possibility of taking one or more of the following measures to reduce dietary exposure to sulfites so that the ADI is not exceeded in the population:

(1) align national legislation with Codex Alimentarius Commission MLs where these are lower;

(2) take action to effectively enforce national MLs;

(3) encourage research on alternative methods of preservation, particularly on applications in which the use of sulfites is responsible for a significant contribution;

(4) take action so that the use of sulfites is reduced in foods where safe alternative solutions are available.

Codex Alimentarius Commission codes of practices for certain groups of food commodities, such as fruit juice, dried fruit and processed meat, could be amended to include suggestions to help countries and the food industry in the implementation of a reduction of the use of sulfites in food.

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Annex 1

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

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
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Annex 2

Acceptable daily intakes, other toxicological information and information on specifications

1. Food additives and ingredients evaluated toxicologically or assessed for dietary exposure

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Asparaginase from *Aspergillus niger* expressed in *A. niger*	N	ADI "not specified"[b] when used in the applications specified and in accordance with good manufacturing practice.
Calcium lignosulfonate (40-65) The suffix (40-65) reflects the weight-average molecular weight range (40 000-65 000) to distinguish it from other calcium lignosulfonates in commerce.	N	ADI of 0-20 mg/kg bw based on a NOEL of 2000 mg/kg bw per day from a 90-day toxicity study and a safety factor of 100. The maximum potential dietary exposure to calcium lignosulfonate (40-65) was low and not expected to exceed 7 mg/kg bw per day from use as a carrier of fat-soluble vitamins and carotenoids in food and supplements.
Ethyl lauroyl arginate	N	ADI of 0-4 mg/kg bw for ethyl lauroyl arginate, expressed as ethyl-*N*-lauroyl-L-arginate HCl, based on a NOAEL of 442 mg/kg bw per day in two reproductive toxicity studies and a safety factor of 100. The Committee noted that some of the estimates of high dietary exposure (greater than 95th percentile) exceeded the ADI, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.
Paprika extract Since the source material and the manufacturing process differ for paprika preparations used as a spice and as a food colour, the name "paprika extract" was adopted for use as a	N, T	The Committee did not allocate an ADI. Concern was expressed as to whether the material tested in the 90-day and long-term studies was representative of all commercial production of paprika extract used as food colour. The fact that the material tested contained less than 0.01% capsaicin and the fact that the Committee did not receive

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
food colour, leaving the term "paprika oleoresin" for use as a spice.		adequate data to establish a limit for capsaicin in the specifications for paprika extract added to this concern. New tentative specifications were prepared, pending receipt of additional information on paprika extract used as food colour, including concentrations of capsaicin (to differentiate from materials used as flavours) and additional information about the composition of batches of extract produced by a variety of manufacturers.
Phospholipase C expressed in *Pichia pastoris*	N	ADI "not specified"[b] when used in the applications specified and in accordance with good manufacturing practice.
Phytosterols, phytostanols and their esters	N	Group ADI of 0-40 mg/kg bw for phytosterols, phytostanols and their esters, expressed as the sum of phytosterols and phytostanols in their free form, based on an overall NOAEL of 4200 mg/kg bw per day to which a safety factor of 100 was applied. The overall NOAEL was identified using the combined evidence from several short-term (90-day) studies of toxicity. The Committee considered the margin between this overall NOAEL and the lowest LOAEL from the 90-day toxicity studies of 9000 mg/kg bw per day as adequate for this overall NOAEL to be used as the basis for establishing an ADI. This conclusion is supported by the results of the available studies of reproductive toxicity. Based on available data, the Committee concluded that dietary exposure to phytosterols and phytostanols would typically be within the ADI range.
Polydimethylsiloxane (PDMS)	R	Temporary ADI of 0-0.8 mg/kg bw for PDMS, based on the previous ADI and applying an additional safety factor of 2. The previously established ADI of 0-1.5 mg/kg bw was withdrawn. Results of studies to elucidate the mechanism and relevance of ocular toxicity observed in the submitted toxicology studies, as well as data on actual use levels in foods, should be provided before the end of 2010. The temporary ADI applies to PDMS that meets the revised specifications prepared.

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Steviol glycosides	R	ADI of 0-4 mg/kg bw expressed as steviol, based on a NOEL of 970 mg/kg bw per day from a long-term experimental study with stevioside (383 mg/kg bw per day expressed as steviol) and a safety factor of 100. The results of the new studies presented to the Committee showed no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. Some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides. The Committee recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.
Sulfites Assessment of dietary exposure		The main contributors to total dietary exposure to sulfites differ between countries owing to differing patterns of use of sulfites in foods and of consumption of foods to which sulfites may be added. Thus, dried fruit, sausages and non-alcoholic beverages were the main contributors of sulfites in some countries, whereas these foods are generally produced without the use of sulfites in other countries. In countries where wine is regularly consumed, it was one of the main contributors to dietary exposure in adults. Dietary exposure in high regular consumers of wine (97.5th percentile) was shown to exceed the ADI for sulfites (0-0.7 mg/kg bw) based on MLs in Codex GSFA, MLs in national legislation or the average concentration determined analytically (about 100 mg/l). In children and teenagers, a significant contribution to mean exposure to sulfites could come from fruit juices and soft drinks (including cordial), sausages, various forms of processed potatoes, dried fruit and nuts. Other significant contributions to dietary exposure in the adult population come from dried fruit, sausages and beer.

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
		The Committee provided recommendations on further relevant actions to be considered by countries and the Codex Alimentarius Commission.

[a] N, new specifications prepared; R, existing specifications revised; T, tentative specifications.

[b] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels 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. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

2. Food additives, including flavouring agents, considered for specifications only

Food additive	Specifications[a]
Canthaxanthin	R
Carob bean gum and carob bean gum (clarified)	R
Chlorophyllin copper complexes, sodium and potassium salts	R
Carbohydrase from *Aspergillus niger* varieties	W
Estragole	W
Fast Green FCF	R
Guar gum and guar gum (clarified)	R
Iron oxides	R
Isomalt	R
Monomagnesium phosphate	N
Patent Blue V	R
Sunset Yellow FCF	R
Trisodium diphosphate	N

[a] N, new specifications prepared; R, existing specifications revised; W, existing specifications withdrawn.

3. Flavouring agents

3.1 *Flavourings evaluated by the Procedure for the Safety Evaluation of Flavouring Agents*

3.1.1 *Aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters*

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
Ethyl (*E*)-2-methyl-2-pentenoate	1815	N	No safety concern
2-Methylbutyl 3-methyl-2-butenoate	1816	N	No safety concern
(±)(*E*,*Z*)-5-(2, 2-Dimethylcyclopropyl)-3-methyl-2-pentenal	1817	N	No safety concern
(*E*,*Z*)-4-Methylpent-2-enoic acid	1818	N	No safety concern
(±)-4-Ethyloctanal	1819	N	No safety concern
(*E*)-Geranyl 2-methylbutyrate	1820	N	No safety concern
(*E*)-Geranyl valerate	1821	N	No safety concern
(*E*)-Geranyl tiglate	1822	N	No safety concern
(*E*)-Citronellyl 2-methylbut-2-enoate	1823	N	No safety concern
(*E*)-Ethyl tiglate	1824	N	No safety concern
(*E*,*Z*)-Geranic acid	1825	N	No safety concern
Prenyl formate	1826	N	No safety concern
Prenyl acetate	1827	N	No safety concern
Prenyl isobutyrate	1828	N	No safety concern
Prenyl caproate	1829	N	No safety concern
(±)-Dihydrofarnesol	1830	N	No safety concern
(*E*,*Z*)-3,7,11-Trimethyldodeca-2,6, 10-trienyl acetate	1831	N	No safety concern
(*E*,*Z*)-Phytol	1832	N	No safety concern
(*E*,*Z*)-Phytyl acetate	1833	N	No safety concern
Structural class II			
Methyl 2-methyl-2-propenoate	1834	N	No safety concern

[a]N, new specifications prepared.

3.1.2 *Aliphatic linear α, β-unsaturated aldehydes, acids and related alcohols, acetals and esters*

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
(*Z*)-2-Penten-1-ol	1793	N	No safety concern
(*E*)-2-Decen-1-ol	1794	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
(*Z*)-Pent-2-enyl hexanoate	1795	N	No safety concern
(*E*)-2-Hexenyl octanoate	1796	N	No safety concern
trans-2-Hexenyl 2-methylbutyrate	1797	N	No safety concern
Hept-*trans*-2-en-1-yl acetate	1798	N	No safety concern
(*E*,*Z*)-Hept-2-en-1-yl isovalerate	1799	N	No safety concern
trans-2-Hexenal glyceryl acetal	1800	N	No safety concern
trans-2-Hexenal propylene glycol acetal	1801	N	No safety concern
cis- and *trans*-1-Methoxy-1-decene	1802	N	No safety concern
(*E*)-Tetradec-2-enal	1803	N	No safety concern
(*E*)-2-Pentenoic acid	1804	N	No safety concern
(*E*)-2-Octenoic acid	1805	N	No safety concern
Ethyl *trans*-2-butenoate	1806	N	No safety concern
Hexyl 2-butenoate	1807	N	No safety concern
Ethyl *trans*-2-hexenoate	1808	N	No safety concern
(*E*,*Z*)-Methyl 2-hexenoate	1809	N	No safety concern
Hexyl *trans*-2-hexenoate	1810	N	No safety concern
Methyl *trans*-2-octenoate	1811	N	No safety concern
Ethyl *trans*-2-octenoate	1812	N	No safety concern
(*E*,*Z*)-Methyl 2-nonenoate	1813	N	No safety concern
Ethyl *trans*-2-decenoate	1814	N	No safety concern

[a] N, new specifications prepared.

3.1.3 *Aliphatic secondary alcohols, ketones and related esters*

Flavouring agent	No.	Specificationsa	Conclusions based on current estimated intake
Structural class I			
Isopropenyl acetate	1835	N	No safety concern
1-Octen-3-yl acetate	1836	N	No safety concern
1-Octen-3-yl butyrate	1837	N	No safety concern
6-Methyl-5-hepten-2-yl acetate	1838	N	No safety concern
3-(Hydroxymethyl)-2-octanone	1839	N	No safety concern
(±)-[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one	1840	N	No safety concern
(±)-*cis*- and *trans*-4,8-Dimethyl-3,7-nonadien-2-ol	1841	N	No safety concern
2,4-Dimethyl-4-nonanol	1850	N	No safety concern
Structural class II			
(±)-1-Hepten-3-ol	1842	N	No safety concern
(*E*,*Z*)-4-Octen-3-one	1843	N	No safety concern
(*E*)-2-Nonen-4-one	1844	N	No safety concern
(*E*)-5-Nonen-2-one	1845	N	No safety concern
(*Z*)-3-Hexenyl 2-oxopropionate	1846	N	No safety concern

Flavouring agent	No.	Specificationsa	Conclusions based on current estimated intake
(±)-*cis*- and *trans*-4,8-Dimethyl-3,7-nonadien-2-yl acetate	1847	N	No safety concern
(*E*)-1,5-Octadien-3-one	1848	N	No safety concern
10-Undecen-2-one	1849	N	No safety concern
8-Nonen-2-one	1851	N	No safety concern

[a] N, new specifications prepared.

3.1.4 *Alkoxy-substituted allylbenzenes present in foods and essential oils and used as flavouring agents*

The Committee concluded that the data reviewed on the six alkoxy-substituted allylbenzenes provide evidence of toxicity and carcinogenicity to rodents given high doses for several of these substances. A mechanistic understanding of these effects and their implications for human risk have yet to be fully explored and will have a significant impact on the assessment of health risks from alkoxy-substituted allylbenzenes at the concentrations at which they occur in food.

Flavouring agent	No.	Specifications[a]
Apiole	1787	N
Elemicin	1788	N
Estragole[b]	1789	N
Methyl eugenol[b]	1790	N
Myristicin	1791	N
Safrole[b]	1792	N

[a] N, new specifications prepared. The specifications monographs will include a statement that the safety evaluation has not been completed.

[b] These compounds were evaluated as flavours at the twenty-fifth meeting of the Committee (Annex 1, reference *56*), with inadequate data to conclude an evaluation.

3.1.5 *Esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids*

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
Methyl hexanoate	1871	N	No safety concern
Hexyl heptanoate	1872	N	No safety concern
Hexyl nonanoate	1873	N	No safety concern
Hexyl decanoate	1874	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Heptyl heptanoate	1875	N	No safety concern
Dodecyl propionate	1876	N	No safety concern
Dodecyl butyrate	1877	N	No safety concern

[a] N, new specifications prepared.

3.1.6 *Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers*

The Committee concluded that the Procedure could not be applied to this group, because of the unresolved toxicological concerns. Studies that would assist in the safety evaluation include investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group.

Flavouring agent	JECFA No.	Specifications[a]
2-Methylfuran	1487	S
2,5-Dimethylfuran	1488	S
2-Ethylfuran	1489	S
2-Butylfuran	1490	S
2-Pentylfuran	1491	S
2-Heptylfuran	1492	S
2-Decylfuran	1493	S
3-Methyl-2-(3-methylbut-2-enyl)-furan	1494	S
2,3-Dimethylbenzofuran	1495	S
2,4-Difurfurylfuran	1496	S
3-(2-Furyl)acrolein	1497	S
2-Methyl-3(2-furyl)acrolein	1498	S
3-(5-Methyl-2-furyl)prop-2-enal	1499	S
3-(5-Methyl-2-furyl)-butanal	1500	S
2-Furfurylidenebutyraldehyde	1501	S
2-Phenyl-3-(2-furyl)prop-2-enal	1502	S
2-Furyl methyl ketone	1503	S
2-Acetyl-5-methylfuran	1504	S
2-Acetyl-3,5-dimethylfuran	1505	S
3-Acetyl-2,5-dimethylfuran	1506	S
2-Butyrylfuran	1507	S
(2-Furyl)-2-propanone	1508	S
2-Pentanoylfuran	1509	S
1-(2-Furyl)butan-3-one	1510	S
4-(2-Furyl)-3-buten-2-one	1511	S
Pentyl 2-furyl ketone	1512	S

Flavouring agent	JECFA No.	Specifications[a]
Ethyl 3-(2-furyl)propanoate	1513	S
Isobutyl 3-(2-furan)propionate	1514	S
Isoamyl 3-(2-furan)propionate	1515	S
Isoamyl 4-(2-furan)butyrate	1516	S
Phenethyl 2-furoate	1517	S
Propyl 2-furanacrylate	1518	S
2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate	1519	S
Furfuryl methyl ether	1520	S
Ethyl furfuryl ether	1521	S
Difurfuryl ether	1522	S
2,5-Dimethyl-3-furanthiol acetate	1523	S
Furfuryl 2-methyl-3-furyl disulfide	1524	S
3-[(2-Methyl-3-furyl)thio]-2-butanone	1525	S
O-Ethyl S-(2-furylmethyl)thiocarbonate	1526	S

[a] S, specifications maintained. The specifications monographs will include a statement that the safety evaluation has not been completed.

3.1.7 *Hydroxy- and alkoxy-substituted benzyl derivatives*

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
4-Hydroxy-3,5-dimethoxy benzaldehyde	1878	N	No safety concern
Vanillin 3-(*l*-menthoxy)propane-1,2-diol acetal	1879	N	No safety concern
Sodium 4-methoxybenzoyloxyacetate	1880	N	No safety concern
Vanillin propylene glycol acetal	1882	N	No safety concern
4-Methoxybenzoyloxyacetic acid	1883	N	No safety concern
Structural class III			
Divanillin	1881	N	No safety concern

[a] N, new specifications prepared.

3.1.8 *Miscellaneous nitrogen-containing substances*

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Structural class II			
Methyl isothiocyanate	1884	N	No safety concern
Ethyl isothiocyanate	1885	N	No safety concern
Isobutyl isothiocyanate	1886	N	No safety concern
Isoamyl isothiocyanate	1887	N	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Isopropyl isothiocyanate	1888	N	No safety concern
3-Butenyl isothiocyanate	1889	N	No safety concern
2-Butyl isothiocyanate	1890	N	No safety concern
4-(Methylthio)butyl isothiocyanate	1892	N	No safety concern
4-Pentenyl isothiocyanate	1893	N	No safety concern
5-Hexenyl isothiocyanate	1894	N	No safety concern
5-(Methylthio)pentyl isothiocyanate	1896	N	No safety concern
6-(Methylthio)hexyl isothiocyanate	1897	N	No safety concern
Structural class III			
Amyl isothiocyanate	1891	N	No safety concern
Hexyl isothiocyanate	1895	N	No safety concern

[a] N, new specifications prepared.

3.1.9 *Monocyclic and bicyclic secondary alcohols, ketones and related esters*

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
Dehydronootkatone	1862	N	No safety concern
Isobornyl isobutyrate	1863	N	No safety concern
l-Bornyl acetate	1864	N	No safety concern
Thujyl alcohol	1865	N	No safety concern
Structural class II			
Vetiverol	1866	N	No safety concern
Vetiveryl acetate	1867	N	No safety concern
3-Pinanone	1868	N	No safety concern
Isobornyl 2-methylbutyrate	1869	N	No safety concern
Verbenone	1870	N	No safety concern

[a] N, new specifications prepared.

3.1.10 *Substances structurally related to menthol*

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
Menthyl valerate	1852	N	No safety concern
2-(*l*-Menthoxy)ethanol	1853	N	No safety concern
l-Menthyl acetoacetate	1854	N	No safety concern
l-Menthyl (*R*,*S*)-3-hydroxybutyrate	1855	N	No safety concern
8-*p*-Menthene-1,2-diol	1860	N	No safety concern
Structural class II			

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
l-Piperitone	1856	N	No safety concern
2,6,6-Trimethylcyclohex-2-ene-1, 4-dione	1857	N	No safety concern
Menthyl pyrrolidone carboxylate	1858	N	No safety concern
3,9-Dimethyl-6-(1-methylethyl)-1, 4-dioxaspiro[4.5]decan-2-one	1859	N	No safety concern
d-2,8-*p*-Menthadien-1-ol	1861	N	No safety concern

[a] N, new specifications prepared.

3.2 *Re-evaluation of safety of certain flavourings*

At the fifty-ninth, sixty-first, sixty-third and sixty-fifth meetings of the Committee (Annex 1, references *160*, *166*, *173* and *178*), only "anticipated" annual volumes of productions were provided for some flavouring agents and used in the MSDI calculation. These volumes were used for expedience in completing a safety evaluation, but the conclusions of the Committee were made conditional pending the submission of actual poundage data.

Actual production volumes were subsequently submitted for all 143 requested flavouring agents and were evaluated by the Committee. The two flavouring substances requiring a re-evaluation were No. 1414, L-monomenthyl glutarate, and No. 1595, 2-isopropyl-*N*,2,3-trimethylbutyramide.

The Committee concluded that the Procedure could not be applied to 2-isopropyl-*N*,2,3-trimethylbutyramide because of evidence of clastogenicity in the presence, but not in the absence, of metabolic activation.

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Ethyl cyclohexanecarboxylate	963	S	No safety concern
10-Hydroxymethylene-2-pinene	986	S	No safety concern
2,5-Dimethyl-3-furanthiol	1063	S	No safety concern
Propyl 2-methyl-3-furyl disulfide	1065	S	No safety concern
Bis(2-methyl-3-furyl) disulfide	1066	S	No safety concern
Bis(2,5-dimethyl-3-furyl) disulfide	1067	S	No safety concern
Bis(2-methyl-3-furyl) tetrasulfide	1068	S	No safety concern
2,5-Dimethyl-3-furan thioisovalerate	1070	S	No safety concern
Furfuryl isopropyl sulfide	1077	S	No safety concern
2-Methyl-3, 5- or 6-(furfurylthio)pyrazine	1082	S	No safety concern
3-[(2-Methyl-3-furyl)thio]-4-heptanone	1085	S	No safety concern
2,6-Dimethyl-3-[(2-methyl-3-furyl) thio]-4-heptanone	1086	S	No safety concern
4-[(2-Methyl-3-furyl)thio]-5-nonanone	1087	S	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
2-Methyl-3-thioacetoxy-4, 5-dihydrofuran	1089	S	No safety concern
4-Hydroxy-4-methyl-5-hexenoic acid gamma- lactone	1157	S	No safety concern
(±) 3-Methyl-gamma-decalactone	1158	S	No safety concern
4-Hydroxy-4-methyl-7-*cis*-decenoic acid gamma-lactone	1159	S	No safety concern
Tuberose lactone	1160	S	No safety concern
Dihydromintlactone	1161	S	No safety concern
Mintlactone	1162	S	No safety concern
Dehydromenthofurolactone	1163	S	No safety concern
(±)-(2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid gamma-lactone	1164	S	No safety concern
2-(4-Methyl-2-hydroxyphenyl)propionic acid gamma-lactone	1167	S	No safety concern
2,4-Hexadien-1-ol	1174	S	No safety concern
(*E*,*E*)-2,4-Hexadienoic acid	1176	S	No safety concern
(*E*,*E*)-2,4-Octadien-1-ol	1180	S	No safety concern
2,4-Nonadien-1-ol	1183	S	No safety concern
(*E*,*Z*)-2,6-Nonadien-1-ol acetate	1188	S	No safety concern
(*E*,*E*)-2,4-Decadien-1-ol	1189	S	No safety concern
Methyl (*E*)-2-(*Z*)-4-decadienoate	1191	S	No safety concern
Ethyl 2,4,7-decatrienoate	1193	S	No safety concern
(±) 2-Methyl-1-butanol	1199	S	No safety concern
2-Methyl-2-octenal	1217	S	No safety concern
4-Ethyloctanoic acid	1218	S	No safety concern
8-Ocimenyl acetate	1226	S	No safety concern
3,7,11-Trimethyl-2,6,10-dodecatrienal	1228	S	No safety concern
12-Methyltridecanal	1229	S	No safety concern
1-Ethoxy-3-methyl-2-butene	1232	S	No safety concern
2,2,6-Trimethyl-6-vinyltetrahydropyran	1236	S	No safety concern
Cycloionone	1239	S	No safety concern
2,4-Dimethylanisole	1245	S	No safety concern
1,2-Dimethoxybenzene	1248	S	No safety concern
4-Propenyl-2,6-dimethoxyphenol	1265	S	No safety concern
erythro- and threo-3-Mercapto-2-methylbutan-1-ol	1289	S	No safety concern
(±)-2-Mercapto-2-methylpentan-1-ol	1290	S	No safety concern
3-Mercapto-2-methylpentanal	1292	S	No safety concern
4-Mercapto-4-methyl-2-pentanone	1293	S	No safety concern
spiro[2,4-Dithia-1-methyl-8-oxabicyclo (3.3.0)octane-3,3•-(1•-oxa-2•-methyl)-cyclopentane]	1296	S	No safety concern
2,3,5-Trithiahexane	1299	S	No safety concern
Diisopropyl trisulfide	1300	S	No safety concern
2-(2-Methylpropyl)pyridine	1311	S	No safety concern
2-Propionylpyrrole	1319	S	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
2-Propylpyridine	1322	S	No safety concern
4-Methylbiphenyl	1334	S	No safety concern
d-3-Carene	1342	S	No safety concern
Farnesene (alpha and beta)	1343	S	No safety concern
1-Methyl-1,3-cyclohexadiene	1344	S	No safety concern
trans-2-Octen-1-yl acetate	1367	S	No safety concern
trans-2-Octen-1-yl butanoate	1368	S	No safety concern
cis-2-Nonen-1-ol	1369	S	No safety concern
(*E*)-2-Octen-1-ol	1370	S	No safety concern
(*E*)-2-Butenoic acid	1371	S	No safety concern
(*E*)-2-Decenoic acid	1372	S	No safety concern
(*E*)-2-Heptenoic acid	1373	S	No safety concern
(*Z*)-2-Hexen-1-ol	1374	S	No safety concern
trans-2-Hexenyl butyrate	1375	S	No safety concern
(*E*)-2-Hexenyl formate	1376	S	No safety concern
trans-2-Hexenyl isovalerate	1377	S	No safety concern
trans-2-Hexenyl propionate	1378	S	No safety concern
trans-2-Hexenyl pentanoate	1379	S	No safety concern
(*E*)-2-Nonenoic acid	1380	S	No safety concern
(*E*)-2-Hexenyl hexanoate	1381	S	No safety concern
(*Z*)-3- and (*E*)-2-Hexenyl propionate	1382	S	No safety concern
2-Undecen-1-ol	1384	S	No safety concern
Dihydronootkatone	1407	S	No safety concern
beta-Ionyl acetate	1409	S	No safety concern
alpha-Isomethylionyl acetate	1410	S	No safety concern
3-(*l*-Menthoxy)-2-methylpropane-1, 2-diol	1411	S	No safety concern
Bornyl butyrate	1412	S	No safety concern
d,*l*-Menthol-(±)-propylene glycol carbonate	1413	S	No safety concern
L-Monomenthyl glutarate	1414	S	No safety concern
L-Menthyl methyl ether	1415	S	No safety concern
p-Menthane-3,8-diol	1416	S	No safety concern
Taurine	1435	S	No safety concern
L-Arginine	1438	S	No safety concern
L-Lysine	1439	S	No safety concern
Tetrahydrofurfuryl cinnamate	1447	S	No safety concern
(±)-2-(5-Methyl-5-vinyltetrahydrofuran-2-yl) propionaldehyde	1457	S	No safety concern
Ethyl 2-ethyl-3-phenylpropanoate	1475	S	No safety concern
2-Oxo-3-phenylpropionic acid	1478	S	No safety concern
2-Oxo-3-phenylpropionic acid sodium salt	1479	S	No safety concern
2-Methyl-3-(1-oxopropoxy)-4H-pyran-4-one	1483	S	No safety concern
4-Allylphenol	1527	S	No safety concern
2-Methoxy-6-(2-propenyl)phenol	1528	S	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Eugenyl isovalerate	1532	S	No safety concern
cis-3-Hexenyl anthranilate	1538	S	No safety concern
Citronellyl anthranilate	1539	S	No safety concern
Ethyl *N*-methylanthranilate	1546	S	No safety concern
Ethyl *N*-ethylanthranilate	1547	S	No safety concern
Isobutyl *N*-methylanthranilate	1548	S	No safety concern
Methyl *N*-formylanthranilate	1549	S	No safety concern
Methyl *N*-acetylanthranilate	1550	S	No safety concern
Methyl *N,N*-dimethylanthranilate	1551	S	No safety concern
N-Benzoylanthranilic acid	1552	S	No safety concern
Trimethyloxazole	1553	S	No safety concern
2,5-Dimethyl-4-ethyloxazole	1554	S	No safety concern
2-Ethyl-4,5-dimethyloxazole	1555	S	No safety concern
2-Isobutyl-4,5-dimethyloxazole	1556	S	No safety concern
2-Methyl-4,5-benzo-oxazole	1557	S	No safety concern
2,4-Dimethyl-3-oxazoline	1558	S	No safety concern
Butyl isothiocyanate	1561	S	No safety concern
Benzyl isothiocyanate	1562	S	No safety concern
Phenethyl isothiocyanate	1563	S	No safety concern
4,5-Dimethyl-2-propyloxazole	1569	S	No safety concern
4,5-Epoxy-(*E*)-2-decenal	1570	S	No safety concern
beta-Ionone epoxide	1571	S	No safety concern
Epoxyoxophorone	1573	S	No safety concern
Ethylamine	1579	S	No safety concern
Propylamine	1580	S	No safety concern
Isopropylamine	1581	S	No safety concern
Isobutylamine	1583	S	No safety concern
sec-Butylamine	1584	S	No safety concern
Pentylamine	1585	S	No safety concern
2-Methylbutylamine	1586	S	No safety concern
Hexylamine	1588	S	No safety concern
2-(4-Hydroxyphenyl)ethylamine	1590	S	No safety concern
1-Amino-2-propanol	1591	S	No safety concern
Butyramide	1593	S	No safety concern
1,6-Hexalactam	1594	S	No safety concern
2-Isopropyl-*N*,2,3-trimethylbutyramide	1595	S	**Further information is needed**
N-Ethyl (*E*)-2,(*Z*)-6-nonadienamide	1596	S	No safety concern
N-Cyclopropyl (*E*)-2,(*Z*)-6-nonadienamide	1597	S	No safety concern
N-Isobutyl (*E,E*)-2,4-decadienamide	1598	S	No safety concern
(±)-*N,N*-Dimethyl menthyl succinamide	1602	S	No safety concern
1-Pyrroline	1603	S	No safety concern
2-Acetyl-1-pyrroline	1604	S	No safety concern
2-Propionylpyrroline	1605	S	No safety concern
Isopentylidene isopentylamine	1606	S	No safety concern
2-Methylpiperidine	1608	S	No safety concern
Triethylamine	1611	S	No safety concern

Flavouring agent	No.	Specifications[a]	Conclusions based on current estimated intake
Tripropylamine	1612	S	No safety concern
N,*N*-Dimethylphenethylamine	1613	S	No safety concern
Trimethylamine oxide	1614	S	No safety concern
Piperazine	1615	S	No safety concern

Annex 3

Further information required or desired

Alkoxy-substituted allylbenzenes present in foods and essential oils and used as flavouring agents (Apiole, JECFA No. 1787; Elemicin, No. 1788; Estragole, No. 1789; Methyl eugenol, No. 1790; Myristicin, No. 1791; Safrole, No. 1792)

There is evidence of toxicity and carcinogenicity to rodents given high doses for several of these substances. A mechanistic understanding of these effects and their implications for human risk have yet to be fully explored and will have a significant impact on the assessment of health risks from alkoxy-substituted allylbenzenes at the concentrations at which they occur in food. Further research is needed to assess the potential risk to human health from low-level dietary exposure to alkoxy-substituted allylbenzenes present in foods and essential oils and used as flavouring agents.

Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (JECFA Nos, Structural Class II: 1487, 1488, 1489, 1490, 1491, 1492, 1493, 1494, 1497, 1499, 1503, 1504, 1505, 1507, 1508, 1509, 1510, 1511, 1513, 1514, 1515, 1516, 1517, 1520, 1521, 1522, 1523, 1524, 1525, 1526; Structural Class III: 1495, 1496, 1498, 1500, 1501, 1502, 1506, 1512, 1518, 1519)

The Committee concluded that the Procedure could not be applied to this group of flavouring agents because of the unresolved toxicological concerns. Studies that would assist in the safety evaluation include investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group of flavours.

2-Isopropyl-*N*,2,3-trimethylbutyramide (JECFA No. 1595)

The Committee concluded that the Procedure could not be applied to 2-isopropyl-*N*,2,3-trimethylbutyramide because of evidence of clastogenicity in the presence, but not in the absence, of metabolic activation. Information

that would assist in resolving the concerns would include data on the potential of this compound to form reactive metabolites and on whether clastogenicity is also expressed in vivo, as well as additional information on the effects found in the kidney (tubular nephrosis, tubular dilatation with granular casts and hyaline droplet formation) at relatively low doses.

Mineral oils (low and medium viscosity), classes II and III

The re-evaluation of the safety of mineral oils (low and medium viscosity), classes II and III, was deferred to a future meeting. The Committee received information from the sponsor that relevant studies are being undertaken and agreed to maintain the temporary ADI until the end of 2009, awaiting additional data to be submitted.

Paprika extract

The Committee requested data on the composition and capsaicin content of batches of paprika extract for use as a colour produced by a variety of manufacturers and information as to whether the material used in the toxicological tests submitted was representative of all the products in commerce. If not, additional toxicological data on representative material would be needed for the evaluation of paprika extract for use as a colour.

Polydimethylsiloxane

The Committee established a temporary ADI for polydimethylsiloxane, pending the results of studies to elucidate the mechanism and relevance of the ocular toxicity and provision of data on actual use levels in foods. The temporary ADI will be withdrawn if the required data are not provided before the end of 2010.

Annex 4

Summary of the safety evaluation of secondary components for flavouring agents with minimum assay values of less than 95%

JECFA No.	Flavouring agent	Minimum assay value (%)	Secondary components	Comments on secondary components
Aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters				
1817	(±)(*E*,*Z*)-5-(2,2-Dimethylcyclopropyl)-3-methyl-2-pentenal	90%	<10% citral	A group ADI of 0-0.5 mg/kg bw, expressed as citral, was established for citral, geranyl acetate, citronellol, linalool and linalyl acetate by the Committee at its twenty-third meeting (Annex, reference *50*). At the sixty-first meeting of the Committee, when citral (No. 1225) was evaluated using the Procedure, citral was concluded to be of no safety concern at current estimated levels of intake as a flavouring agent, and the ADI was maintained (Annex 1, reference *166*).
Aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters				
1800	*trans*-2-Hexenal glyceryl acetal	86%	8% 3-hexenal glyceryl acetal; 1% hexanal glyceryl acetal	3-Hexenal glyceryl acetal and hexanal glyceryl acetal are expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to the corresponding aldehydes and alcohols, followed by complete metabolism in the fatty acid pathway or the tricarboxylic cycle. They do not present a safety concern at current estimated levels of intake of the flavouring agent.

1810	Hexyl *trans*-2-hexenoate	92%	6-8% hexyl *trans*-3-hexenoate	Hexyl *trans*-3-hexenoate is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to 3-hexenoic acid and hexanol, followed by complete metabolism in the fatty acid pathway or the tricarboxylic cycle. It does not present a safety concern at current estimated levels of intake of the flavouring agent.
1811	Methyl *trans*-2-octenoate	90%	5-6% methyl *trans*-3-octenoate	Methyl *trans*-3-octenoate is expected to share the same metabolic fate as the primary substance, i.e. hydrolysis to 3-octenoic acid and methanol, followed by complete metabolism in the fatty acid pathway or the tricarboxylic cycle. It does not present a safety concern at current estimated levels of intake of the flavouring agent.
Aliphatic secondary alcohols, ketones and related esters				
1839	3-(Hydroxymethyl)-2-octanone	90%	7% 3-methylene-2-octanone	3-Methylene-2-octanone (No. 1149) was evaluated by the Committee at its fifty-ninth meeting (Annex 1, reference *160*) and was concluded to be of no safety concern at current estimated levels of intake as flavouring agent.

1850	2,4-Dimethyl-4-nonanol	84%	6.6% 2,6,8-trimethyl-6-hydroxy-4-nonanone; 6.5% *cis*-2,6,8-trimethyl-5-nonen-4-one; 2.6% *trans*-2,6,8-trimethyl-5-nonen-4-one	2,6,8-Trimethyl-6-hydroxy-4-nonanone, *cis*-2,6,8-trimethyl-5-nonen-4-one and *trans*-2,6,8-trimethyl-5-nonen-4-one are expected to share the same metabolic fate as the primary substance, i.e. reduction of the ketone followed by glucuronic acid conjugation. They do not present a safety concern at current estimated levels of intake of the flavouring agent.
Hydroxy- and alkoxy-substituted benzyl derivatives				
1879	Vanillin 3-(*l*-menthoxy)-propane-1,2-diol acetal	94%	2-3% vanillin	An ADI of 0-10 mg/kg bw was established for vanillin by the Committee at its eleventh meeting (Annex, reference *14*). At the fifty-seventh meeting of the Committee, when vanillin (No. 889) was evaluated using the Procedure, vanillin was concluded to be of no safety concern at current estimated levels of intake as a flavouring agent, and the ADI was maintained (Annex 1, reference *154*).
1881	Divanillin	91%	5-7% vanillin	See above
1882	Vanillin propylene glycol acetal	79%	18-20% vanillin	See above

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, including flavouring agents, with a view to recommending acceptable daily intakes (ADIs) and to preparing specifications for identity and purity.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation and assessment of intake of food additives (in particular, flavouring agents). A summary follows of the Committee's evaluations of technical, toxicological and intake data for certain food additives (asparaginase from *Aspergillus niger* expressed in *A. niger*, calcium lignosulfonate (40–65), ethyl lauroyl arginate, paprika extract, phospholipase C expressed in *Pichia pastoris*, phytosterols, phytostanols and their esters, polydimethylsiloxane, steviol glycosides and sulfites [assessment of dietary exposure]) and 10 groups of related flavouring agents (aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters; aliphatic linear α,β-unsaturated aldehydes, acids and related alcohols, acetals and esters; aliphatic secondary alcohols, ketones and related esters; alkoxy-substituted allylbenzenes present in foods and essential oils and used as flavouring agents; esters of aliphatic acyclic primary alcohols with aliphatic linear saturated carboxylic acids; furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers; miscellaneous nitrogen-containing substances; monocyclic and bicyclic secondary alcohols, ketones and related esters; hydroxy- and alkoxy-substituted benzyl derivatives; and substances structurally related to menthol).

Specifications for the following food additives were revised: canthaxanthin; carob bean gum and carob bean gum (clarified); chlorophyllin copper complexes, sodium and potassium salts; Fast Green FCF; guar gum and guar gum (clarified); iron oxides; isomalt; monomagnesium phosphate; Patent Blue V; Sunset Yellow FCF; and trisodium diphosphate. Re-evaluation of flavouring agents for which estimated intake was based on anticipated poundage data was carried out for 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595) and L-monomenthyl glutarate (No. 1414).

Annexed to the report are tables summarizing the Committee's recommendations for intakes and toxicological evaluations of the food additives considered.

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