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EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

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

Food and Agriculture Organization of the United Nations

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Sixty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Geneva, 19–28 June 2007

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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 and contaminants. WHO Food Additives Series, No. 59, in press.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications. FAO JECFA Monographs 4, 2007.

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 Geneva from 19 to 28 June 2007. The meeting was opened by Susanne Weber-Mosdorf, Assistant Director General, World Health Organization (WHO), on behalf of the Directors General of WHO and the Food and Agriculture Organization of the United Nations (FAO). Mrs Weber-Mosdorf acknowledged the important role the work of the Committee plays in the establishment of international food safety standards and its contribution to sustainable development. She informed the Committee of the six-point agenda that the new Director General of WHO, Dr Margaret Chan, has proposed for the organization. This agenda also refers to using evidence to define strategies and measure results, and in this context the work of the Committee is an important contribution to the goals of WHO. Mrs Weber-Mosdorf informed the Committee that the 50th World Health Assembly approved an increased budget for the areas of food safety and nutrition and for public health and the environment, which illustrated the importance the Member States give to these work areas. She emphasized that FAO and WHO consider the provision of scientific advice in food safety an important and core activity.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the sixty-eighth meeting of JECFA 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. Professor Gary Williams declared an interest in steviol glycosides and did not participate in the discussions. 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 sodium iron ethylenediaminetetraacetic acid (EDTA) and of acidified sodium chlorite (ASC).

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 67 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 and contaminants, in particular additional considerations on the assessment of dietary exposure to flavouring agents (section 2);

— to undertake toxicological evaluations of certain food additives and contaminants (sections 3 and 4 and Annex 2);

— to review and prepare specifications for certain food additives (section 3 and Annex 2).

2.1 Modification of the agenda

When discussing the compound "cyclotetraose", the Committee considered this name inappropriate and decided to name the compound cyclotetraglucose. In addition to evaluating the crystalline cyclotetraglucose, the Committee received and evaluated information relating to a syrup of cyclotetraglucose.

The flavouring ethyl 2-methyl-3,4-pentadienoate (No. 353) was added to the agenda for evaluation of a 92-day dietary study, as requested by the Committee at its fifty-first meeting (Annex 1, reference *137*). The flavourings *cis*- and *trans*-2-isopropyl-4-methyl-1,3-dioxolane (No. 1713) and *cis*- and *trans*-2-isobutyl-4-methyl-1,3-dioxolane (No. 1714) were not considered, as these were identified as identical to the flavourings isobutanal propyleneglycol acetal (No. 1748) and isovaleraldehyde propylene glycol acetal (No. 1732), respectively.

Furfural was considered for re-evaluation of specifications only, pending availability of additional data for safety assessment. The evaluation of estragole was deferred to a future meeting, pending submission of data requested for the assessment of safety and specifications.

The food additives polyvinyl alcohol and pectins and the flavouring monomenthyl L-glutarate (No. 1414) were added to the agenda, for revision of specifications.

2.2 Report from the thirty-ninth Codex Committee on Food Additives (CCFA) and the first 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 thirty-ninth session of CCFA. As this was the first meeting after the split of the Codex Committee on Food Additives and Contaminants (CCFAC) into two separate committees, amendments to the terms of reference and the risk analysis principles were proposed. CCFA proposed about 320 provisions for food additives for adoption by the Codex Alimentarius Commission. Twenty JECFA specifications were also proposed for adoption as Codex specifications, and five were proposed to be revoked. CCFA agreed on a revised guideline for the use of flavourings for adoption at step 5 of the Codex procedure, pending further elaboration on how to address a list of biologically active substances with proposed maximum levels (MLs) in foods. Finally, CCFA agreed on a list of food additives proposed for evaluation by JECFA.

The Chairman of the Codex Committee on Contaminants in Food (CCCF), Mr Ger de Peuter, summarized for the Committee key discussions of the first session of CCCF based on the assessments provided by JECFA. MLs were proposed for tin in canned foods and beverages for adoption by the Codex Alimentarius Commission, and a draft ML was proposed for 3-monochloropropanediol in acid-hydrolysed vegetable protein-containing products. CCCF decided to postpone work on aflatoxin and ochratoxin pending the outcome of the JECFA assessments. CCCF also agreed on a list of priority substances to be evaluated by JECFA.

2.3 Principles governing the toxicological evaluation of compounds on the agenda

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in 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* and *184*), 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 No-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL): use in JECFA assessments

In its safety assessment of food additives and contaminants in food, the Committee identifies no-observed-effect levels (NOELs) in establishing acceptable or tolerable intakes. The Committee has until now used the term NOEL defined as follows (Annex 1, reference *76*):

NOEL: *The greatest concentration or amount of an agent, found by study or observation, that causes no detectable,* ***usually adverse****, alteration of morphology, functional capacity, growth, development, or lifespan of the target.* [emphasis added]

The Committee noted that other national and international risk assessment bodies, including the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), differentiate between the term NOAEL, to identify that an effect is considered adverse, and NOEL, for effects not considered adverse.

In WHO Environmental Health Criteria, No. 170, *Assessing the human health risks of chemicals: derivation of guidance values for health-based exposure limits* (*2*), these terms are defined as follows:

No-observed-adverse-effect level (NOAEL): *greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target organism under defined conditions of exposure....*

No-observed-effect level (NOEL): *greatest concentration or amount of a substance, found by experiment or observation, that causes no alteration of morphology, functional capacity, growth, development, or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.*

Taking into account common practice in other risk assessment bodies and in order to harmonize with JMPR, the Committee decided to use the term NOAEL when the relevant effect at the next higher dose is considered

adverse. If such an effect is not considered adverse, then the term NOEL will be used. This includes assessments where no effects were observed at the highest dose tested. In such cases, the highest dose tested will be taken as the NOEL (see examples of the enzymes asparaginase, isoamylase and phospholipase A1 evaluated at this meeting).

The same approach will be used by the Committee with respect to the terms lowest-observed-effect level (LOEL) and lowest-observed-adverse-effect level (LOAEL).

The Committee noted that it was not possible to implement this decision for the evaluation of flavouring agents at this meeting and that the term NOEL is used according to the previous practice.

The Committee emphasized that this decision does not entail any change in its evaluation practice. It is merely harmonizing the terminology used to differentiate between observed effects and observed adverse effects. Hence, this decision has no impact on any of the previous evaluations made by this Committee.

2.5 The safety evaluation of flavouring agents

2.5.1 *Dietary exposure estimates for use in the Procedure*

Introduction

JECFA employs the maximized survey-derived intake (MSDI) method as a measure of dietary exposure for use in the Procedure for the Safety Evaluation of Flavouring Agents (the Procedure), as first described in the report of the forty-sixth meeting (Annex 1, reference *122*). The MSDI provides an estimate of the mean exposure of consumers to a flavouring agent, which can then be 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 disappearing into the food supply per year in specific regions, currently Europe, the United States of America (USA) and Japan,[1] and on the assumption that 10% of the relevant population would consume the foods containing the flavouring agent. The MSDI includes a correction for the proportion of the annual production volume reported. At this meeting, a correction factor of 0.8 was applied to the annual production volume reported in recent surveys in Europe (*3*),[2] Japan (*4*) and the USA (*5*), and the populations of consumers used in the

[1] Data from Japan were available for the first time at the present (sixty-eighth) meeting.

[2] Previous meetings had assumed that 60% of the annual production volume was reported for the USA and 80% for Europe.

MSDI calculations were 32×10^6 in Europe, 13×10^6 in Japan and 28×10^6 in the USA.

The Committee considered issues related to the dietary exposure to flavouring agents at its forty-fourth, forty-sixth, forty-ninth, fifty-fifth, sixty-third, sixty-fifth and sixty-seventh meetings (Annex 1, references *116*, *122*, *131*, *149*, *173*, *178* and *184*). The estimation of dietary exposures of consumers to flavouring agents based on annual production volume data was considered to be a practical and realistic approach for the average (mean) consumer; it includes an assumption that 10% of the population are consumers of the flavouring agent. Further consideration of potential health risks for high consumers of specific flavouring agents was recommended in cases where it was anticipated that there may be high use levels in specific foods, but low predicted dietary exposures when calculated by the MSDI method. The specific concern of the Committee was that the distribution of use levels for some flavouring agents may be uneven across different food categories and within food categories, and this uneven distribution cannot be taken into account in the MSDI. At its sixty-fifth meeting, the Committee proposed that an ad hoc Working Group be convened to consider an additional estimate of exposure to complement the MSDI based on use levels recommended by the industry.

Development of the single-portion exposure technique

Having examined a published analysis of data on over 800 flavouring agents (*6*), the ad hoc Working Group noted at the sixty-seventh meeting that dietary exposures derived using use levels in foods, determined to be generally recognized as safe (GRAS) by the Flavor and Extract Manufacturers Association (FEMA) in the USA, and the possible average daily intake (PADI) or modified theoretical added maximum daily intake (mTAMDI) methods could be several orders of magnitude higher than the MSDI. Analysis of the safety implications showed that in the great majority of cases, the differences between these estimates would not have affected the conclusions reached by the Committee on those flavouring agents using the Procedure. The Committee agreed to explore an additional new method of dietary exposure assessment (since termed the single-portion exposure technique, or SPET) based on the daily consumption of a single portion of food containing the flavouring agent, in recognition that the alternative methods that assume daily consumption of large portions of several food categories containing the flavouring agent (PADI, TAMDI, mTAMDI) were overly conservative.

The SPET provides a dietary exposure estimate based on use levels recommended by the industry and aims to represent the chronic dietary exposure for a regular consumer who consumes daily a specific food product containing the flavouring agent of interest. The SPET identifies the single food category

containing the flavouring agent of interest that is likely to contribute the highest dietary exposure based on a "standard portion" size.[3] The standard portion is taken to represent the mean food consumption amount for consumers of that food category, assuming daily consumption over a long period of time. The standard portion does not reflect high food consumption amounts reported in national dietary surveys for the food category and is therefore a more realistic prediction of long-term consumption patterns.

Development of criteria to identify flavouring agents of potential concern

At its sixty-seventh meeting, the Committee proposed to focus on a limited number of flavouring agents having production volume data at the lower and upper ends of the distribution. The Committee noted that although the discrepancies between different methods were greatest for low reported production volume data, there was no clear cut-off value that could be used to define a "low production volume data" flavouring agent. An annual production volume of less than 10 kg in each specific region was selected by the Committee as a value to identify flavouring agents that might have limited food applications and for which there might be greater uncertainty about their use and distribution across various food categories.

Data on use levels for flavouring agents

Data were received by the Committee from the International Organization of the Flavor Industry (IOFI) for 57 of the 168 flavouring agents evaluated at the meeting. These flavouring agents were selected from the eight groups for further assessment of dietary exposure based on use levels recommended by the industry. One of the following two criteria was used for the selection:

1. flavouring agents with production volume of less than 10 kg per year; or
2. flavouring agents with production volume that resulted in MSDI values of more than one third of the relevant TTC value. This assumes that high consumers of such flavouring agents may possibly have dietary exposures close to the TTC. The cut-off was chosen because of a known ratio, derived from national dietary surveys, of mean to high-percentile food consumption amounts of 1:3.

Of the 57 flavouring agents selected, 44 had low production volumes, 5 had high production volumes and 8 had intermediate production volumes. Overall, of the 57 flavouring agents assessed, 42 were in structural class I, 6 in class II and 9 in class III.

[3] The standard portion sizes used in the assessment were made available to the public as well as the industry in the report of the sixty-seventh meeting of the Committee (Annex 1, reference *184*).

IOFI identified companies that had submitted relevant data in recent production surveys (2004–2006) and contacted them to provide data on recommended use levels for the 57 flavourings. Use levels were reported in 28 food groups and 116 food subgroups across the three regions. Weighted average use levels for each food category were calculated by IOFI from the individual responses from the different companies that produced each flavouring agent. The data received were considered to be of greater relevance than the FEMA use levels for flavouring agents for the purpose of using the SPET because they identified only those food categories where the flavouring agent was reported to be added and recommended levels of use. These types of data were not previously accessible to this Committee. A comparison of the submitted use level data for flavouring agents with published FEMA use levels indicates that use levels were in most cases similar; however, as expected, flavouring agents were listed in fewer food categories than in the FEMA use levels.[4]

Comparison of dietary exposure estimates from the SPET with the MSDI and application to the Procedure

The SPET was used to estimate dietary exposure for the 57 flavouring agents. The single food categories leading more frequently to the highest potential dietary exposure for flavouring agents from a single standard portion were non-alcoholic drinks (15 cases), fine bakery wares (9 cases), soups (7 cases), processed fruit, particularly fruit jellies (5 cases), and sauces (4 cases). For the remaining 17 flavouring agents, one of various other food categories—for example, snacks, milk products, processed vegetable products, confectionary, decorations, bread or meat and fish products—led to the highest dietary exposure from a single standard portion. No other food categories were identified as the source of the highest dietary exposure from a single food category.

In general, the estimated dietary exposure using the SPET was up to several orders of magnitude higher than that calculated by the MSDI for any of the three geographic regions for which production volume data were available (Europe, Japan and USA), as shown in Table 1. Although the dietary exposure estimates from the SPET and MSDI were not correlated, the ratio of the SPET to the MSDI was strongly correlated to the MSDI when plotted on a logarithmic scale. There was a tendency for the discrepancy between the dietary exposure estimates from the SPET and the MSDI to be greater for flavouring agents with low production volume. In four cases, however, the MSDI method provided a higher dietary exposure estimate than that calculated using the SPET (one flavouring agent with a low production volume, and three with high production volumes).

[4] Where IOFI cannot provide recommended use levels for a flavouring agent in a specific food category, the published FEMA use levels could be used in the SPET, providing the industry can confirm its use in this food category.

Table 1
Single-portion exposure technique (SPET) and maximum survey-derived intake (MSDI) dietary exposure estimates

JECFA No.	FEMA No.	Structural class	Criteria[a]	TTC (μg/person per day)	Food group with highest SPET (daily portion considered)	SPET (μg/person per day)	Highest MSDI (μg/person per day)	SPET/MSDI (rounded)	Above TTC (MSDI or SPET)
1624	4164	I	LP	1800	Fruit-flavoured, Carbonated; Carbonated, Other Types (300 g)	300	0.12	2500	
1626	4112	I	LP	1800	Drink Mix Powders; Fruit-flavoured, Carbonated; Carbonated, Other Types (300 g)	600	0.12	5000	
1636	4036	I	LP	1800	Fruit-flavoured, Carbonated; Non-carbonated, Less than 10% Fruit Juice; Carbonated, Other Types (300 g)	15	0.01	1500	
1653	2393	I	IP	1800	Drink Mix Powders; Fruit-flavoured, Carbonated; Carbonated, Other Types; Non-cola Diet Beverages; Non-carbonated, Less than 10% Fruit Juice; Carbonated Cola Beverages (300 g)	1500	17.12	90	
1674	4167	I	LP	1800	Drink Mix Powders; Fruit-flavoured, Carbonated (300 g)	60	0.01	6000	
1695	4042	I	LP	1800	Meat-type Gravies; Soup Mixes Used as Sauces; Cream- and Cream-like-based Sauces (30 g)	0.9	0.01	90	

1701	4029	I	IP	1800	Non-cola Diet Beverages; Carbonated, Other Types (300 g)	15	14.02	1
1712	4048	I	LP	1800	Fruit-flavoured, Carbonated; Non-carbonated, Less than 10% Fruit Juice; Carbonated, Other Types (300 g)	600	0.01	60 000
1737	4384	I	LP	1800	Drink Mix Powders; Fruit-flavoured, Carbonated; Non-carbonated, Less than 10% Fruit Juice; Drinks and Ades (>10% Fruit Juice); Carbonated, Other Types (300 g)	120	0.03	4000
1739	4368	I	LP	1800	Non-carbonated, Less than 10% Fruit Juice; Drinks and Ades (>10% Fruit Juice) (300 g)	900	0.26	3460
1764	4137	III	LP	90	Unspecified Meat Products; Processed Crustaceans, Fabricated/Frozen (100 g)	40	1.07	40
1740	4382	I	LP	1800	Drink Mix Powders; Non-carbonated, Less than 10% Fruit Juice (300 g)	1050	0.53	1980
1747	4381	I	LP	1800	Non-carbonated, Less than 10% Fruit Juice; Carbonated, Other Types (300 g)	1500	0.53	2830
1767	4232	III	LP	90	Meat-type Gravies; Tomato-based Sauces; Tomato Sauce/ Paste/Puree (30 g)	60	0.12	500

Table 1 *(continued)*

1779	4267	III	HP	90	Flavoured Snack Items; Filled Crackers; Unspecified (30 g)	90	30.58	3	
1754	4278	II	IP	540	Cakes; Cookies and Graham Crackers; Unspecified Baked Goods (80 g)	80	31.35	3	
1756	4280	II	IP	540	Cakes; Cookies and Graham Crackers; Unspecified Baked Goods; Bread Sticks and Pretzels (80 g)	80	143.57	0.6	
1711	4099	I	IP	1800	Drink Mix Powders; Fruit-flavoured, Carbonated; Beers, Ales, and Other Malt Beverages (300 g)	3000	135.38	20	SPET
1621	4356	I	HP	1800	Drink Mix Powders; Fruit-flavoured, Carbonated; Carbonated, Other Types (300 g)	1500	602.37	2	
1619	4262	I	LP	1800	Concentrated Tomato Base (200 g)	1	0.1	10	
1622	4046	I	LP	1800	Concentrated Tomato Base (200 g)	1	0.12	10	
1623	4351	I	LP	1800	Bread Crumbs/Cubes/Breadings (50 g)	12.5	0.03	420	
1630	4165	I	LP	1800	Drink Mix Powders (300 g)	60	0.86	70	
1632	4361	I	LP	1800	Drink Mix Powders (300 g)	90	0.03	3000	
1634	4358	I	LP	1800	Fruit-type Sweet Sauces and Toppings (125 g)	375	0.79	470	
1651	4268	I	LP	1800	Cough Drops; Hard Candies (30 g)	600	0.61	980	

1655	2392	I	IP	1800	Cakes; Bread Sticks and Pretzels (80 g)	1280	574.36	2	
1656	2394	I	HP	1800	Cakes; Bread Sticks and Pretzels (80 g)	720	1020.43	0.7	
1662	4333	I	LP	1800	Yogurt (200 g)	20	0.24	80	
1666	4159	III	LP	90	Other Canned or Frozen Types (200 g)	40	1.28	30	
1670	4157	I	LP	1800	Cakes (80 g)	16	0.06	270	
1678	4172	I	LP	1800	Cakes (80 g)	36	0.12	300	
1685	4108	I	LP	1800	Non-carbonated, Less than 10% Fruit Juice (300 g)	15	0.43	30	
1688	4181	I	LP	1800	Carbonated, Other Types (300 g)	150	0.01	15 000	
1689	4182	I	LP	1800	Ready-to-Eat Vegetable Products (200 g)	150	0.01	15 000	
1690	4166	I	LP	1800	Ready-to-Eat Vegetable Products (200 g)	10	0.01	1000	
1692	4183	I	LP	1800	Dried Vegetable Mixes (200 g)	200	0.01	20 000	
1700	4073	II	LP	540	Unspecified Soups (200 g)	20	0.08	250	
1703	4282	I	LP	1800	Bread Crumbs/Cubes/Breadings (50 g)	100	0.12	830	
1714	4286	I	LP	1800	Beers, Ales, and Other Malt Beverages (300 g)	1500	10.54	140	
1721	4186	I	IP	1800	Milk- and Cream-based Sauces (80 g)	1800	714.16	3	SPET
1728	4098	I	LP	1800	Drink Mix Powders (300 g)	600	0.03	20 000	
1743	4373	I	LP	1800	Non-chocolate Flavoured Milk (200 g)	200	0.53	380	
1751	4275	II	LP	540	Non-alcoholic Beverages, Unspecified (300 g)	30	1.84	20	

Table 1 *(continued)*

1753	4277	II	IP	540	Cookies and Graham Crackers (80 g)	112	78.24	1	
1758	4035	II	LP	540	Dry Soup Mixes; Bouillon, All Types (200 g)	400	0.03	13 330	
1759	3817	III	LP	90	Dry Soup Mixes; Bouillon, All Types (200 g)	2	8.13	0.3	
1760	4064	III	LP	90	Cookies and Graham Crackers (80 g)	26.4	0.01	2640	
1762	4318	III	LP	90	Yogurt (200 g)	80	0.01	8000	
1768	4233	III	LP	90	Tomato-based Sauces (30 g)	60	0.24	250	
1776	4309	III	HP	90	Frostings, Icings, Glazes (35 g)	70	34	2	
1780	4132	I	LP	1800	Flavoured Jellies (125 g)	625	0.03	20 830	
1781	4131	I	LP	1800	Flavoured Jellies (125 g)	625	0.03	20 830	
1782	4134	I	LP	1800	Flavoured Jellies (125 g)	625	6.97	90	
1783	4133	I	LP	1800	Flavoured Jellies (125 g)	625	0.12	5210	
1785	4187	I	LP	1800	Other Canned or Frozen Types (200 g)	100	0.01	10 000	
–	3408	I	HP	1800	Cakes; Bread Sticks and Pretzels (80 g)	16	1875.73	0.01	MSDI

[a] LP = Low Production data; HP = MSDI above one third of TTC; IP = Intermediate Production data.

To assess the potential for differences in outcomes using different dietary exposure assessment methods as part of the Procedure, the higher of the dietary exposure estimates from either the MSDI or the SPET calculations for each flavouring agent was compared with the TTC for each structural class. The MSDI was just above the TTC in one case (class I, high production volume); the SPET estimate was approximately 2-fold greater than the TTC in one case and equalled the TTC in one case (the latter two had intermediate production volumes and were class I). Only the two flavouring agents where the SPET estimate was equal to or exceeded the TTC would have had a different route through the Procedure; in other words, these two compounds were further evaluated through step A4 or B4, as appropriate, rather than being judged "*not expected to be of safety concern at the current levels of intake*" at the previous step (as they would have been if the MSDI had been used). Moreover, the eventual decision was the same when the margin of safety between the SPET and the NOEL was assessed at the further step. This analysis indicated that it would not be necessary to re-evaluate flavouring agents that have already been assessed using the Procedure.

The potential for the higher dietary exposure estimates obtained with the SPET to affect the answers at step 2 ("*Can the substance be predicted to be metabolized to innocuous products?*") and at step B5 ("*Do the conditions of use result in an intake greater than 1.5 μg/day?*") was also investigated by the Working Group. It was concluded that when a higher dietary exposure was obtained with the SPET, it would not significantly affect the answer at step 2 of the Procedure, whereas it may affect the answer to question B5, noting that a very limited number of flavouring agents proceeded to this step.

Conclusion

The Committee noted that the use levels submitted by IOFI were considered to be of greater relevance than the published FEMA use levels for flavouring agents for use in the SPET dietary exposure estimate, because IOFI identified only those food categories where the flavouring agent was added while maintaining confidentiality of actual levels used by individual companies.

On the basis of the analysis undertaken for this meeting, the Committee concluded that the MSDI and SPET dietary exposure estimates provide different and complementary information. The SPET takes account of food consumption patterns and use levels of flavouring agents and is considered to provide an estimate of dietary exposure for a regular daily consumer of a specific food product containing the flavouring agent. The MSDI is considered to provide an estimate of the dietary exposure of the flavouring agent for an average consumer; because it is based on the reported annual production volume, it cannot take use patterns into account. The Committee noted that the addition

of the SPET dietary exposure estimate to the relevant step in the Procedure would be likely to lead to a more extended evaluation in only a limited number of cases. The Committee noted that this analysis indicated that it would not be necessary to re-evaluate flavouring agents that have already been assessed using the Procedure.

Prior to a final decision on the addition of the SPET dietary exposure estimate to the Procedure, the Committee agreed at this meeting to repeat the assessment of a selected number of flavouring agents using both the MSDI and SPET dietary exposure estimates for evaluation at the next meeting. It was noted that the three cases where the dietary exposure estimate exceeded the TTC (derived from the MSDI or the SPET) were not low production volume flavouring agents, for which concern had previously been expressed by the Committee. The Committee recognized a need to consider dietary exposures for regular consumers of intermediate and high production volume flavouring agents with different use patterns. A sample representative of different levels of production volume and use patterns reported for flavouring agents will be selected for this assessment, ensuring that flavouring agents from each class and group are included, the list not limited to those scheduled for evaluation at the next meeting. Another outcome of the future work will be the further development of suitable criteria for selecting flavouring agents where additional information on added use levels recommended by the industry is required for use in the SPET, prior to evaluation.

2.5.2 *Consideration of combined dietary exposure estimates for flavouring agents*

At the present meeting, the Committee reconsidered the justification and approach taken to consider the combined dietary exposure estimates for flavouring agents that had been evaluated as members of the same group.

Considerations of combined dietary exposures were introduced when the Procedure was used for the first time at the forty-sixth meeting to evaluate esters of allyl alcohol. Allyl esters are hydrolysed to a common toxic metabolite, allyl alcohol. Combined dietary exposure assessment was based on the total per capita estimated daily intake of allyl alcohol that would arise from the simultaneous consumption of the 21 allyl esters in the group, and this estimate was compared with the acceptable daily intake (ADI) that had been established previously for allyl alcohol. The same meeting also undertook combined dietary exposure assessments of the esters of ethyl and isoamyl alcohols by combining the MSDI estimates of the different agents leading to the same common metabolite, ethanol or isoamyl alcohol, respectively, and comparing the result with the endogenous synthesis of ethanol and the ADI for isoamyl alcohol, respectively. That meeting also raised the issue of combining dietary exposure estimates from across different groups where

assessment of these data and the toxicological profile warranted a combined appraisal. The groups evaluated at subsequent meetings contained more diverse structures, and considerations of combined dietary exposures were usually based on addition of the dietary exposure estimates for the different agents in each structural class and comparison of the combined dietary exposure estimates with the relevant thresholds for the structural classes.

JECFA has now evaluated almost 1800 flavouring agents. The Committee has generally considered the combined dietary exposure estimates within each group of flavouring agents. However, a number of short-chain alcohols and acids are predicted to be common metabolites of a diverse range of flavouring agents evaluated in different groups at different meetings. The present meeting evaluated additional flavouring agents for groups that had been considered at previous meetings. The combination of dietary exposure estimates for different flavouring agents evaluated at different meetings highlighted the need to reconsider the rationale for the approach that has been adopted.

The present meeting discussed the likelihood that concurrent dietary exposures might occur and the rationale for simple dose addition in the combined dietary exposure assessments.

The phrase "in the unlikely event that all foods containing all flavouring agents were consumed simultaneously on a daily basis" has been used in reports as a caveat to allow for the low probability of simultaneous dietary exposure to all the substances in a group. The dietary exposure estimate for each flavouring agent assumes that consumers represent 10% of the total population. If each substance in a group of flavouring agents were to be present in a different food item/product, the proportion of the population that would have simultaneous dietary exposure to *n* substances would be 0.1^n. In reality, the proportion of the population having simultaneous dietary exposure would not be as low as this, because mixtures of flavouring agents are added to the same food/product to produce the desired flavour. Nevertheless, the likelihood of the "unlikely event" will be diminishingly small for the larger groups of flavouring agents.

Dose addition would be an appropriate approach for combined dietary exposure assessments if the individual substances share common toxicokinetics and/or toxicodynamics, as was the case for the allyl esters evaluated when the Procedure was first used.

Flavouring agents are metabolized by a variety of pathways, including those important in intermediary metabolism, such as β-oxidation, or by enzymes involved in foreign compound metabolism, such as esterases, alcohol and aldehyde dehydrogenases, cytochrome P450 and conjugation with

glucuronic acid or glutathione. Although some pathways are saturable at high substrate concentrations—for example, conjugation with glutathione or sulfate—the metabolic pathways have high capacities and would not be saturated by combined exposures to flavouring agents. In consequence, as has been stated in the various reports, saturation of metabolism would not arise from combined dietary exposures to substances sharing the same metabolic pathway. Many groups of flavouring agents include a range of different chemical structures and molecules with multiple sites of metabolism, so that combining all substances in a group as representing the mass of potential substrates for a single metabolic pathway is not logical.

A common adverse effect could arise from simultaneous exposure to flavouring agents, owing to their having the same, but unknown, site and mode of action at high doses. Flavouring agents with diverse structures would be unlikely to show common adverse effects. Dose addition would be logical for flavouring agents that produce the same common metabolite, providing that the metabolite is the active toxic entity, as is the case for esters of allyl alcohol. In most cases, the common metabolite is of low potential toxicity and often is endogenous, so that dose addition would not provide an indication of possible risk. Dose addition would be logical for substances that are members of a homologous series—for example, when each flavouring agent in the series has the same functional group(s) and they differ only in the length of side-chains. Dose addition on a weight basis would assume that all members of the series show equal potency on a weight basis, whereas dose addition on a molar basis would allow for molecular weight differences.

The Committee recommended that the assessment of combined dietary exposure for flavouring agents at future meetings should be undertaken for:

1. *Flavouring agents that share a common metabolite.* The dietary exposures for up to five substances with the highest estimated dietary exposures should be added and expressed on the basis of the common metabolite, assuming complete conversion to the common metabolite (in the absence of quantitative metabolic data). The resulting combined dietary exposure estimate should be evaluated in relation to the known toxicity of the common metabolite. In the absence of toxicity data on the common metabolites, the combined dietary exposure should be evaluated in relation to the threshold for the structural class of the common metabolite.

2. *Flavouring agents that are members of a homologous series.* The dietary exposures for up to five flavouring agents with the highest estimated dietary exposures should be added on a molar basis and evaluated in relation to the toxicity of the most potent member of those substances combined or, in the absence of such data, in relation to the most potent member of

the series. In the absence of toxicity data on any member of the series, the combined dietary exposure on a weight basis should be evaluated in relation to the threshold for the relevant structural class.

2.6 Extension of an existing ADI to substances obtained from different sources and/or by different manufacturing processes

A recurring question facing the Committee is whether an ADI allocated to an additive obtained from a specific source material and/or by a specific manufacturing process can be applied to encompass similar additives obtained by other means or from other sources. The agenda for the present meeting included some additives where this question arose. The Committee therefore considered the possibility of elaborating principles or guidelines for evaluations in this area.

At previous meetings, the Committee has evaluated several food additives containing the same chemical entity as the functional component in relation to its food additive use but obtained from different source materials and/or different manufacturing processes. One such example is colours containing β-carotene, which may be obtained by extraction from vegetables, algae or a genetically modified microorganism (GMM) or produced by chemical synthesis.[5]

Depending on the substance in question and information received, the Committee has reached various conclusions in its evaluations.

A guiding principle in the safety evaluation of food additives has been that the material tested toxicologically is representative of the material of commerce. To this end, specifications have been established primarily to reflect substances that have been toxicologically tested and secondarily to cover as far as possible products commercially available in the market. When additives containing the same chemical entity or entities as the functional component are produced from different sources or by different methods of manufacture, possibly leading to substantial differences in composition, it has been found necessary to prepare more than one specifications monograph, as was the case for the above-mentioned β-carotene-containing additives.

In order to answer the question of whether it is possible to apply an existing ADI to an additive from a new source or novel method of manufacture (the "new product"), the Committee considered that it is necessary to compare the source, method of manufacture and composition of the new product with those of the product that was tested toxicologically and for which the ADI was originally allocated (the "old product").

[5] Eighteenth JECFA (Annex 1, reference *35*): β-Carotene (synthetic); forty-first JECFA (Annex 1, reference *107*): Carotenes (vegetable) and Carotenes (algae); fifty-seventh JECFA (Annex 1, reference *154*): β-Carotene from *Blakeslea trispora*.

In addition to the source, the composition of a product is commonly related to the manufacturing process. Typical methods of manufacture are chemical synthesis, extraction from natural source materials and production by a microorganism (with or without genetic modification). All of these may result in different residues and impurities that have to be taken into account.

The content of the functional component(s) is often low in products obtained by extraction of natural source materials, whereas the content is normally high in products obtained by chemical synthesis. It is evident that a product with a low content of the functional component contains significant amounts of other substances (e.g. components resulting from the source material or from an organism used in its production).

The Committee recommended that the following stepwise procedure be adopted to determine whether a new product might be included in a previously allocated ADI:

1. Information on the manufacture and composition of both the new and the old products should be collated and compared, and any major differences or significant lack of information identified.

2. Data from the first step might be used to determine whether

   a) the new product is sufficiently similar to the old product to be included in the ADI,

   b) it is impossible to include the new product in the ADI because of substantial compositional differences between the two products, or

   c) it may be possible to include the new product in the ADI provided that additional information is received by the Committee.

   In all cases, both the nature and amount of any new by-products/solvent residues or other contaminants need to be considered. If the content of the functional component(s) of the additive is high and any new contaminants would be present in only minute amounts, consideration of the intake of the additive and consequential intake of the minor contaminant may indicate whether the presence is of safety concern.

3. The specifications should include information on source, manufacture and composition in order to reflect materials covered by the evaluation. In particular, where indicated for toxicological reasons, criteria/limits for specific components should be included in the specifications.

2.7 Guidelines for the safety evaluation of enzymes produced by genetically modified microorganisms

At its sixty-fifth meeting (Annex 1, reference *178*), the Committee concluded that guidelines need to be developed on the safety evaluation of enzymes

produced by GMMs. These guidelines should address the information considered essential for different enzyme preparations and the details considered necessary for molecular characterization of the producing microbial strain to allow adequate assessment of its safety.

At the present meeting, the Committee reviewed comments on these considerations submitted by the Enzyme Technical Association and the Association of Manufacturers and Formulators of Enzyme Products. The Committee also noted the ongoing international initiatives to elaborate guidelines for the safety evaluation of enzymes (including those from GMMs) and microorganisms intended for food applications. These documents are expected to be finalized in the near future. The Committee recommended that the subject of guidelines for the safety evaluation of enzymes produced by GMMs be addressed at a future meeting.

2.8 Specifications for substances with both food additive and flavouring agent use

At its fifty-ninth meeting, the Committee concluded that it was not desirable to develop specifications for flavouring agents separate from those specifications it has developed for other uses (Annex 1, reference *160*). In such cases, the material used for flavouring purposes should comply with the specifications in the food additive format. It was decided that the list of specifications for flavouring agents should simply make reference to the full specifications monograph. At the present meeting, it was noted that this procedure has not been followed.

The Committee reviewed the decision of the fifty-ninth meeting and decided that substances with additive uses in addition to use as a flavouring agent should have specifications in both the food additives format and the flavouring agent format. However, the specifications in the two formats should contain comparable purity requirements.

The Committee agreed to review, at a future meeting, whether any substantial differences exist between sets of specifications and to make revisions as necessary.

2.9 Withdrawal of certain food additive specifications

2.9.1 *Anisyl acetone*

The Committee reviewed the tentative specifications in the traditional food additives format for anisyl acetone. These specifications list flavouring agent as the only functional use.

This substance was considered as a flavouring agent at the fifty-seventh meeting of the Committee under the name 4-(*p*-methoxyphenyl)-2-butanone

(Annex 1, reference *154*), and new specifications were prepared in the flavouring agent format.

The Committee decided to withdraw the tentative specifications for anisyl acetone in the traditional food additives format, as these are superseded by the specifications for 4-(*p*-methoxyphenyl)-2-butanone in the flavouring agent format.

2.9.2 *Furfural*

The Committee reviewed the tentative specifications for furfural in the traditional food additives format. These list extraction solvent as the only functional use. Furfural also has specifications in the flavouring agent format under No. 450.

At its fifty-first meeting, the Committee concluded that the only functional use of furfural was as a flavouring agent (Annex 1, reference *137*). Although it elaborated new specifications in the flavouring agent format, the existing tentative specifications for the extraction solvent use were retained. However, the Committee still has no information that furfural has any direct food use other than as a flavouring agent, and therefore it decided to withdraw the tentative food additives format specifications for furfural as an extraction solvent.

2.9.3 *Zeaxanthin-rich extract from* Tagetes erecta

During its sixty-third meeting (Annex 1, reference *173*), the Committee prepared tentative specifications for zeaxanthin-rich extract from *Tagetes erecta* and requested information on the non-zeaxanthin components in total carotenoid content of the extract, as well as information on the non-carotenoid components. The Committee did not receive sufficient information to allow it to remove the tentative designation. Furthermore, the Committee was informed that additional information would not be available in the near future, and therefore it decided to withdraw the existing tentative specifications. The Chemical and Technical Assessment on zeaxanthin synthetic and zeaxanthin-rich extract prepared for the sixty-third JECFA (Annex 1, reference *173*) and revised during the sixty-seventh JECFA (Annex 1, reference *184*) was maintained.

3. Specific food additives (other than flavouring agents)

The Committee evaluated four food additives 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 *Acidified sodium chlorite*

Explanation

Acidified sodium chlorite (ASC) possesses antimicrobial properties and is intended for use primarily as a spray or dipping solution for poultry, meats, vegetables, fruits and seafoods. It is also used in poultry chilling water. ASC is produced by the addition of a food-grade acid (e.g. citric acid, phosphoric acid, hydrochloric acid, malic acid or sodium hydrogen sulfate) to an aqueous solution of sodium chlorite. Combining the acid with sodium chlorite results in conversion of chlorite to chlorous acid, which can subsequently form a mixture of chlorite, chlorate, chlorine dioxide and chloride. The Committee has not previously evaluated ASC or sodium chlorite used in the preparation of ASC. Among the acids mentioned above that may be used in the preparation of ASC, sodium hydrogen sulfate has not been evaluated by the Committee.

ASC was evaluated by the Committee at its present meeting as requested by CCFAC at its thirty-eighth session (*7*). The Committee was asked to provide a risk assessment for ASC for use in food contact (as a processing aid).

The safety of chlorite, chlorate, chlorine dioxide and chloride in drinking-water has previously been assessed by WHO. WHO established tolerable daily intakes (TDIs) of 0.03 mg/kg body weight (bw) for both chlorite and chlorate.

Residual chlorine dioxide is lost by evaporation; hence, chlorite, chlorate and chloride are the principal residues expected. The chloride generated as a result of treatment with ASC is negligible compared with the chloride already present in food. The use of chlorine to disinfect water supplies results in formation of by-products such as trihalomethanes. However, chlorine dioxide acts as an oxidizing agent and therefore does not form trihalomethanes or by-products other than chlorite and chlorate ions. The residues of the food-grade acids (e.g. phosphate, citrate, malate, sulfate) are commonly present in food and have previously established ADIs. Therefore, the Committee focused the toxicological evaluation on ASC, chlorite and chlorate.

The Committee received a submission containing published information on ASC, including studies on a germicidal product developed for clinical uses and on sodium chlorite. Additional information identified by a literature search relates to the safety, absorption, distribution, metabolism and excretion or biochemistry of ASC, chlorite or chlorate in relation to animal, human or in vitro models.

Chemical and technical considerations

Sodium chlorite is marketed in two forms, as a solid containing approximately 80% sodium chlorite and as an aqueous solution. Sodium chlorite is manufactured by reducing sodium chlorate, chemically or electrochemically, in the presence of hydrochloric acid to produce chlorine dioxide. Chlorine dioxide is then reacted with hydrogen peroxide in aqueous sodium hydroxide to yield a solution containing 30–50% sodium chlorite, which is subsequently dried to a solid or further diluted to get an aqueous solution.

ASC is intended for use as part of an integrated approach designed to control microbial loads on foodstuffs. ASC solution acts to reduce the number of pathogens (e.g. *Escherichia coli* O157:H7, *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes*), as well as, to a somewhat lesser extent, spoilage bacteria found on the surface of foods during processing. The solution is applied onto the surface of different types of fresh and processed foods at a concentration range of 50–1200 mg sodium chlorite/l. Fresh and processed fruits and vegetables are subjected to a water rinse after ASC application followed by a 24-h withholding time (for cut produce only). Treatment of whole or parts of poultry carcasses, sausages or delicatessen meats (cold cuts) is carried out by spraying or dipping prior to or after chilling. ASC is also used to treat pre-chilling and chilling water, into which poultry carcasses are submerged, at relatively low levels (i.e. 50–150 mg sodium chlorite/l). Poultry and meat products are not rinsed subsequent to treatment.

Toxicological data

The available database to assess the safety of ASC and its residues chlorite and chlorate has limitations, and few of the studies have been conducted to the current standards expected for regulatory submissions. The studies on ASC related to a germicidal product, and some of these involved parenteral administration. The Committee considered that although not directly relevant to oral exposure, these studies provided useful supplementary information that did not raise concern about the use of acidified chlorite as a processing aid. In many of the studies in which chlorite or chlorate was administered in drinking-water, the information provided was insufficient to derive an accurate estimation of the dose received by the animals.

Chlorite and chlorate are rapidly absorbed into the plasma and distributed throughout the body, with the highest concentrations in plasma. They are excreted primarily in the urine in the form of chloride, with lesser amounts of chlorite and chlorate. However, the extent to which these are formed as chemical degradation products prior to absorption or as a result of biotransformation was unclear. There was some indication of metabolism to chloroform, but the data were inadequate to evaluate or to use in the safety assessment.

ASC and chlorite are of moderate acute toxicity, but only limited data were available on chlorate. Studies conducted with sodium chlorite in a number of species demonstrated that the most consistent finding is oxidative stress associated with changes in erythrocytes. This observation was also supported by a number of biochemical studies conducted in vitro. Some studies have indicated that the effect may be related to a reduction in serum glutathione levels, thus reducing the body's ability to protect the erythrocytes from the effects of sodium chlorite. Other studies have indicated that sodium chlorite may cause damage to the erythrocyte membrane. For effects on erythrocytes, the lowest LOAEL of 19 mg/kg bw per day, expressed as chlorite, was derived from a 13-week gavage study in rats in which the NOAEL was 7.4 mg/kg bw per day, expressed as chlorite. Studies on sodium chlorate in a number of species showed some effects on haematological parameters and on body weight gain.

Although sodium chlorate has also been reported to have effects on erythrocytes, changes in thyroid histology (e.g. colloid depletion, hypertrophy, incidence and severity of hyperplasia) and in thyroid hormones were the most sensitive effects observed in rats administered sodium chlorate in drinking-water for 21 or 90 days. Male rats were more sensitive than females, as is commonly seen with substances that affect thyroid function. In one of the two available 90-day studies, thyroid hypertrophy and decreased colloid were observed in male rats given sodium chlorate at drinking-water concentrations

of 1 mg/l as chlorate (equivalent to about 0.1 mg chlorate/kg bw per day) and above. In general, effects including incidence and severity of follicular cell hyperplasia were dose related and more consistently observed at chlorate doses of 75 mg/kg bw per day and above.

Sodium chlorite was not carcinogenic following a number of long-term studies, although these were not conducted to current standards. The International Agency for Research on Cancer (IARC) concluded in 1991 that sodium chlorite was not classifiable with respect to carcinogenicity to humans. Sodium chlorite has given positive results in some, but not all, in vitro genotoxicity assays and in one of the two available in vivo mouse micronucleus assays involving intraperitoneal administration. Negative results were obtained in several in vivo assays, for induction of bone marrow micronuclei, chromosomal aberrations and sperm-head abnormalities, involving oral administration of sodium chlorite to mice.

Sodium chlorate has recently been tested for carcinogenicity in rats and mice under the United States National Toxicology Program; results of these studies were not available at the time WHO set the TDI for chlorate. There was no evidence of carcinogenic activity in male B6C3F1 mice and equivocal evidence in female mice based on marginally increased incidences of pancreatic islet neoplasms. Sodium chlorate produced positive results in some in vitro assays, but not for induction of bone marrow micronuclei or chromosomal aberrations following oral administration to mice. There was some evidence of carcinogenic activity in male and female F344/N rats based on increased incidences of thyroid gland neoplasms. The incidence of thyroid gland follicular hypertrophy was enhanced compared with control groups at doses lower than those resulting in increased tumour incidences and was significantly greater than control in the male rats at all tested doses. Therefore, the lowest dose, equivalent to approximately 5 mg/kg bw per day, expressed as chlorate, was the LOAEL. Because a NOAEL was not identified in the study, the Committee decided to apply a benchmark dose (BMD) approach to derive a point of departure on the dose–response curve (see Annex 1, reference *176*). The United States Environmental Protection Agency (USEPA) BMD software version 1.4.1 was used for modelling the rat thyroid gland follicular cell hypertrophy data. The calculated BMD_{10} values for a 10% increase in thyroid gland follicular cell hypertrophy in the male rats ranged from 1.9 to 5.9 mg/kg bw per day, expressed as chlorate. The 95% lower confidence limit for the BMD ($BMDL_{10}$) values ranged from 1.1 to 4.4 mg/kg bw per day, expressed as chlorate. The Committee used the lowest $BMDL_{10}$ of 1.1 mg/kg bw per day, expressed as chlorate, which was derived from the model giving the best fit to the data, for its further evaluation of chlorate. For female rats, the BMD_{10} values ranged from 4.7 to 12.6 mg/kg bw per day, and the $BMDL_{10}$ values ranged from 3.0 to 6.4 mg/kg bw per day.

Based on the negative in vivo genotoxicity data and the nature of the histopathological observations, the Committee concluded that a non-genotoxic mode of action was likely for the induction of thyroid tumours by sodium chlorate. This mode of action is likely to be mediated via decreased serum thyroid hormones, leading to increased release of thyroid stimulating hormone (TSH) and consequent stimulation of thyroid cell proliferation and thyroid gland growth, which can lead to thyroid tumours in rodents.

In addition to thyroid carcinogenesis, this mode of action raises concerns about possible neurodevelopmental effects, since thyroid hormone status is critical to normal brain development.

Reproductive toxicity studies have shown no adverse effects of ASC or sodium chlorite on fertility. A multigeneration study of reproduction and developmental neurotoxicity was available in which sodium chlorite was administered to rats in drinking-water at a concentration of 35, 70 or 300 mg/l. Published information indicated that the highest dose tested resulted in effects on body weight in both sexes of the parental generation and a range of effects in the offspring, including decreased body weight, changes in haematological parameters and a decrease in maximum startle response amplitude at postnatal day (PND) 24, but not at PND 60. A small but statistically significant decrease in maximum startle response amplitude was also reported at the middle dose at PND 24. The Committee considered that this observation was attributable to perturbed habituation in the control animals. Other effects observed in the offspring of the high-dose group (i.e. reduced absolute brain weight and slight delays in attainment of sexual maturity) could be attributable to reduced body weight. In addition, a USEPA toxicological review of chlorine dioxide and chlorite cited data contained only in the unpublished original study report showing reduced absolute and relative liver weights in the F0 females and F1 males and females of the high-dose group and in the F0 females and F1 males of the mid-dose group. The Committee concluded that the low dose in this study, equivalent to 3 mg/kg bw per day, expressed as chlorite, was the NOAEL.

Administration of sodium chlorate to pregnant rats resulted in no maternal or developmental effects at the highest tested dose of 1000 mg/kg bw per day. Neurodevelopmental end-points were not investigated in this study, and no multigeneration study was available.

Special studies on nephrotoxicity, immune function and sperm quality in vivo indicated that such effects would not be critical to the safety assessment.

Studies in healthy adult male volunteers lasting up to 12 weeks showed no clear treatment-related effects on blood, urinalysis or physical examination

at doses of sodium chlorite and sodium chlorate estimated to be in the region of 0.036 mg/kg bw per day, expressed as chlorite or chlorate.

Assessment of dietary exposure

The Committee estimated potential dietary exposure on the basis of the residual concentrations of chlorite and chlorate, as reported in the submitted data for raw products of three food categories (meat and meat products, including poultry; fish and fish products; and fruits and vegetables) that had been treated with ASC solution. The treatment was at the proposed use level of 1200 mg sodium chlorite/l and under optimum conditions to fulfil the technological purpose (with sufficient time of spray or immersion and drip with water wash and holding time).

The available data showed that residues of chlorite and chlorate in most foods treated with ASC declined to levels below the limits of detection (LODs) with time (after treatment, rinsing and a holding period).

The occurrence data used in the calculation of dietary exposure estimates were as follows: for meat and meat products, 0.1 mg/kg for both chlorite and chlorate; for seafood and freshwater fish, 0.01 mg chlorite/kg and 0.1 mg chlorate/kg; for fruits and vegetables, 0.01 mg chlorite/kg for all fruits and vegetables, except for leafy vegetables (0.23 mg chlorite/kg), and 0.01 mg chlorate/kg.

Dietary exposures were then estimated using the 13 Global Environment Monitoring System Food Contamination Monitoring and Assessment Programme (GEMS/Food) Consumption Cluster Diets[6] and food consumption data from the European Union (EU) countries for the general population. The Committee noted that the estimates were highly conservative, as it was assumed that all the treated foods will be consumed daily over a lifetime and that all treated foods consumed contain the maximum residual level of chlorite and chlorate.

International mean dietary exposures were estimated to be 0.2–0.7 μg/kg bw per day for chlorite and 0.1–0.6 μg/kg bw per day for chlorate for the 13 GEMS/Food Consumption Cluster Diets, if a body weight of 60 kg is assumed. National estimates for EU countries of mean to 95th percentile daily dietary exposures in the general population were 0.9–3 μg/kg bw for chlorite and 0.3–0.6 μg/kg bw for chlorate.

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

Evaluation

The Committee concluded that the available toxicological data were sufficient to assess the safety of ASC by setting ADIs for chlorite and chlorate.

For chlorite, the Committee established an ADI of 0–0.03 mg/kg bw on the basis of the NOAEL of 3 mg/kg bw per day for reduced liver weight of F0 females and F1 males and females in a two-generation reproductive study in rats and a safety factor of 100 to allow for inter- and intraspecies variability. This ADI is supported by the results of studies in human volunteers showing no adverse effects at this intake.

For chlorate, the Committee concluded that the most sensitive effects were changes to the thyroid gland of male rats. Rats are considered to be highly sensitive to the effects of agents that disrupt thyroid hormone homeostasis. The Committee considered that humans are likely to be less sensitive than rats to these effects and that a safety factor for interspecies variation was not required. However, the Committee noted deficiencies in the database, particularly with respect to investigation of possible neurodevelopmental effects. The Committee therefore established an ADI of 0–0.01 mg/kg bw for chlorate on the basis of the $BMDL_{10}$ of 1.1 mg/kg bw per day for non-neoplastic effects on the thyroid of male rats in a recent carcinogenicity study, a safety factor of 10 to allow for intraspecies variability and an additional factor of 10 to allow for the deficiencies in the database.

The Committee noted that the occurrence data submitted for chlorite and chlorate, determined using good manufacturing practice for ASC-treated foods, were sufficient to be used in the assessment. These occurrence data were used with national diet data for EU countries and the 13 GEMS/Food Consumption Cluster Diets in a dietary exposure scenario whereby all treated food categories consumed contained chlorite and chlorate at the maximum residual concentrations.

For chlorite, a dietary exposure of 3 μg/kg bw per day could be taken to represent high consumers, including children. For chlorate, a dietary exposure of 0.6 μg/kg bw per day could be taken to represent high consumers, including children.

The Committee concluded that the present conservative estimates of mean and high-level dietary exposure to chlorite and chlorate represented up to 10% of the ADIs. The Committee noted that these estimates were compatible with the exposure allocated to other sources within the WHO drinking-water guidelines for chlorite and chlorate.

Because ASC is a surface treatment, the residue level will be proportional to the surface/volume ratio. Therefore, the current evaluation relies on the submitted protocols, and dietary exposure should be re-estimated if new usages are introduced (e.g. ground beef).

The Committee noted that the use of ASC does not replace the need for good hygienic practices in handling and processing of food.

A toxicological monograph on ASC and new specifications for sodium chlorite and sodium hydrogen sulfate were prepared. A Chemical and Technical Assessment for ASC was prepared.

3.1.2 *Asparaginase from* Aspergillus oryzae *expressed in* Aspergillus oryzae

Explanation

At the request of CCFAC at its thirty-eighth session (*7*), the Committee evaluated the enzyme asparaginase (L-asparagine amidohydrolase; EC 3.5.1.1), which it had not evaluated previously. Asparaginase hydrolyses the amide in the amino acid L-asparagine to the corresponding acid, resulting in L-aspartate (aspartic acid) and ammonia. It is to be used in the manufacture of dough-based products and processed potato products, where asparaginase is added prior to heat treatment of these products with the intention of reducing acrylamide formation.

Genetic modification

The asparaginase enzyme preparation under evaluation is produced by submerged fermentation of *Aspergillus oryzae* carrying a gene encoding asparaginase from *A. oryzae*. The host organism *A. oryzae* is not pathogenic and has a long history of use in food. The specific host strain used, *A. oryzae* BECh2, and the production strain derived therefrom, *A. oryzae* pCaHj621/BECh2#10, were considered to constitute a safe strain lineage, given the genetic modifications that resulted in the removal of genes involved in the synthesis of aflatoxins and cyclopiazonic acid and a reduction in the potential to produce other secondary metabolites (3-β-nitropropionic acid and kojic acid). Furthermore, the expression plasmid was fully characterized, as known deoxyribonucleic acid (DNA) sequences were used in the construction and the DNA derived from *A. oryzae* was limited to the asparaginase coding sequence. The plasmid does not contain antibiotic resistance genes, nor does it contain any unidentified DNA or DNA sequences that would result in the production of toxic substances. Asparaginase expressed by the production strain has no significant amino acid sequence homology with known allergens and toxins. A test batch of the asparaginase enzyme preparation was shown not to contain 3-β-nitropropionic acid or kojic acid.

Chemical and technical considerations

Asparaginase is produced by submerged fed-batch pure culture fermentation of the *A. oryzae* pCaHj621/BECh2#10 production strain. Asparaginase is secreted into the fermentation medium, from which it is recovered and concentrated. It is subsequently stabilized, formulated and standardized with water, glycerol, sodium benzoate and potassium sorbate. The asparaginase enzyme preparation conforms to 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*). The enzyme preparation is free from the production organism.

The recommended use level of asparaginase in the manufacture of dough-based products and processed potato products is up to 2500 asparaginase units/kg processed food. The addition of asparaginase prior to heat treatment of these products is intended to reduce the formation of acrylamide, which is normally formed as a reaction product between asparagine and reducing sugars when food products are baked or fried. The heat processing steps will inactivate the enzyme. It is to be noted that the effectiveness of the asparaginase enzyme preparation in reducing acrylamide formation was not evaluated by the Committee.

Toxicological data

Toxicological studies were performed with an asparaginase liquid enzyme concentrate (LEC). The Committee noted that the materials added to the asparaginase LEC for stabilization, formulation and standardization either have been evaluated previously by the Committee or are common food constituents and do not raise safety concerns.

In a 13-week study of toxicity in rats, no significant treatment-related effects were seen when the LEC was administered by oral gavage at doses up to and including 880 mg of total organic solids (TOS)/kg bw per day. Therefore, 880 mg TOS/kg bw per day, the highest dose tested, was taken to be the NOEL. The LEC was not mutagenic in an assay for mutagenicity in bacteria in vitro and was not clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro.

Assessment of dietary exposure

Based on a maximum mean daily consumption of 960 g of processed foods (cereals, roots and tubers; GEMS/Food Consumption Cluster Diet B) by a 60-kg adult and on the assumptions that the enzyme is used at the maximum recommended use level and that all TOS originating from the enzyme preparation remain in the final products, the dietary exposure would be 0.4 mg TOS/kg bw per day.

Evaluation

Comparing the conservative exposure estimate with the NOEL from the 13-week study of oral toxicity, the margin of safety is 2200. The Committee allocated an ADI "not specified" for asparaginase from this recombinant strain of *A. oryzae*, 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.

3.1.3 *Carrageenan and processed* Eucheuma *seaweed*

Explanation

Carrageenan is a sulfated galactose polymer with an average molecular weight well above 100 kilodaltons (kDa). It is derived from several species of red seaweeds of the class Rhodophyceae. It has no nutritive value and is used in food preparation for its gelling, thickening and emulsifying properties. The three main copolymers in carrageenan are designated as *iota* (ι), *kappa* (κ) and *lambda* (λ), depending on the number and location of the sulfate moieties on the hexose backbone. Processed *Eucheuma* seaweed is a semi-refined form of carrageenan and has been derived from either *E. cottonii* or *E. spinosum*.

Carrageenan was reviewed by the Committee at its thirteenth, seventeenth, twenty-eighth, fifty-first and fifty-seventh meetings (Annex 1, references *19*, *32*, *66*, *137* and *154*). At its twenty-eighth meeting, the Committee established an ADI "not specified" on the basis of the results of a number of toxicological studies on carrageenans obtained from various sources. Processed *Eucheuma* seaweed was reviewed by the Committee at its thirtieth, thirty-ninth, forty-first, forty-fourth, fifty-first and fifty-seventh meetings (Annex 1, references *73*, *101*, *107*, *116*, *137* and *154*). At its fifty-first meeting, the Committee concluded that the toxicities of processed *Eucheuma* seaweed and carrageenan were sufficiently similar for the ADI "not specified" for carrageenan to be extended to a temporary group ADI including processed *Eucheuma* seaweed, pending clarification of the significance of the promotion of colon cancer observed in experiments in rats. At its fifty-seventh meeting, the Committee established a group ADI "not specified" for the sum of carrageenan and processed *Eucheuma* seaweed, as the Committee considered that the intakes of carrageenan and processed *Eucheuma* seaweed from their use as food additives were of no safety concern.

At its present meeting, the Committee was requested by CCFAC to review all data necessary for toxicological re-evaluation, including specific data relevant to the safety assessment for infants aged 0–6 months, from exposure through infant formulas (*7*).

At the present meeting, the Committee reviewed data published since the fifty-seventh meeting in 2001 and other data of specific relevance to the safety assessment for infants. In response to the Committee's request for further data, a toxicological dossier on carrageenan and processed *Eucheuma* seaweed was submitted. In addition, a search of the scientific literature was conducted for the years 2000–2007.

The Committee also considered some new information related to the specifications.

Toxicological data

There was a lack of clarity regarding the types of carrageenan used in some published studies and whether they relate to food-grade carrageenan, which has an average molecular weight well above 100 kDa, or to poligeenan, which has an average molecular weight of 20–30 kDa and is also known as degraded carrageenan. Poligeenan has been widely used as an inflammatory and adjuvant agent in experimental models for investigation of immune processes. Very little is known about the possible degradation of food-grade carrageenan to lower molecular weight species and possible subsequent effects, although some evidence indicates that gastrointestinal metabolism of carrageenan by acid digestion and bacterial degradation to lower molecular weight components might occur. At its fifty-first meeting, the Committee concluded that such breakdown is probably of limited toxicological significance since, if native carrageenan were sufficiently degraded to cause ulceration or tumour growth, this would be detected in feeding studies. Some bacteria are known to hydrolyse carrageenan, resulting in low molecular weight forms. These bacteria, however, are of marine origin, and it is not known if the human microbial flora can perform similar hydrolysis reactions.

Intestinal absorption studies are lacking both for humans and for animals, in vitro and in vivo. At its thirteenth meeting, the Committee commented that little carrageenan is absorbed when ingested by several animal species; subsequently, at its seventeenth meeting, the Committee commented that high molecular weight carrageenan is probably not absorbed. The European Commission's Scientific Committee on Food more recently concluded that it could not be excluded that carrageenan might be absorbed by the immature gut and that absorbed material might affect the immune system in the infant. No new data have been published addressing this issue.

Of the information reviewed previously by the Committee, all animal experiments apart from one in baboons were performed in adult animals and not in suckling infants, which limits their usefulness for the safety evaluation of carrageenan for infants. From the absence of effects in the study in infant baboons fed infant formula containing carrageenan, a NOEL of 1220 mg/l in

formula was identified, equivalent to 432 mg/kg bw per day. However, in this study, the colon was fixed in 10% buffered formalin, and this does not enable identification of mast cells that would be present if an inflammatory process had been initiated. Mast cells of the gastrointestinal tract mucosa can be visualized only using special fixation techniques, because they differ from other mast cells with respect to the spatial arrangement of glycosaminoglycan and protein in their granules.

One new study conducted in mice showed that carrageenan enhanced the tumorigenicity of a carcinogen, *N*-methyl-*N*-nitrosourea, confirming the results of studies previously evaluated by the Committee at its fifty-seventh meeting.

The Committee noted at its fifty-first and fifty-seventh meetings that proliferative effects on the gastrointestinal tract were reported in a number of studies of rats fed dietary concentrations of 2.6% carrageenan, equivalent to 1300 mg/kg bw per day, or greater. No effects were observed in rats at carrageenan concentrations of up to 1.5% in the diet, equivalent to 750 mg/kg bw per day. Proliferative and inflammatory effects were observed in one new study in mice administered *lambda*-carrageenan in the drinking-water at concentrations of 1% and 4% (equivalent to approximately 1100 and 3500 mg/kg bw per day, respectively). A NOAEL was not identified in the mouse study, but the LOAEL, expressed on a body weight basis, was similar to that in the rat.

Effects on the gastrointestinal tract were not reported in a recent 90-day dietary study in rats with food-grade *kappa*-carrageenan with a low molecular weight tail fraction (7% below 50 kDa). No effects were observed at the highest dietary concentration of 50 000 mg/kg, which was equivalent to 3394 mg/kg bw per day. As with the baboon study referred to above, this study would not have identified mast cells.

A number of in vitro mechanistic studies were available. These were of limited value for the safety assessment of dietary carrageenan. Both activation and suppression of the immune system have been reported, apparently involving both nonspecific (macrophage) and specific (lymphocyte) immune responses. Studies evaluating the possible effects on the immune system were limited, in that they used either systemically administered carrageenan or lower molecular weight forms of carrageenan. There was little information with respect to possible effects on the immune system in humans following oral exposure to food-grade carrageenan.

Presumed mechanisms of intestinal injury following oral administration of carrageenan in rodents include damage via free oxygen radicals. Activation of thymidine kinase in epithelial cells of the gut as a sign of increased cell

turnover has also been considered. It is still unclear whether the low absorption of carrageenan via the intestinal tract could affect the immunity of the host. It is also unclear whether absorption may be greater in the neonate, during weaning and in adults and children following allergic reactions and episodes of gastrointestinal disease.

No reports have been identified that address the particular question of effects on the immature intestine and immunity in experimental models or in prospectively designed human studies.

Data submitted summarizing customer complaint records for cow's milk- and soy-based infant formulas with and without carrageenan content did not reveal statistical differences between these groups with respect to blood in stool or upper respiratory tract infections. The Committee noted that these records did not relate to hydrolysed protein- and/or amino acid-based liquid formulas and that such reports would be unlikely to reveal subtle adverse effects. One epidemiological study indicated an association between consumption of carrageenan and incidence of mammary cancer. The Committee concluded that these data did not support a causal relationship because of limitations in the methodology and lack of adjustments for acknowledged risk factors for mammary carcinoma.

Assessment of dietary exposure

The draft Codex infant formula standard (*8*), in section 4.1.7, proposes the following maximum levels in the product ready for consumption:

- 0.03 g/100 ml for regular milk- and soy-based liquid formulas;
- 0.1 g/100 ml for hydrolysed protein- and/or amino acid-based liquid formulas.

The consumption of formula by infants can be calculated based on the caloric requirement of 125 kcal/kg bw per day and on a content of 0.8 kcal/g in formula in order to provide a realistic estimate of dietary exposure to carrageenan.

The average daily exposure to carrageenan from liquid infant formulas was estimated to be 47 mg/kg bw per day for milk- and soy-based formulas (0.03% carrageenan) and 160 mg/kg bw per day for hydrolysed protein- and/or amino acid-based liquid infant formulas (0.1% carrageenan). These exposure estimates apply to infants fed exclusively on formula.

The Committee also estimated exposure to carrageenan of infants of 12 months of age, based on a survey in France showing that consumption of formula represents 13.7% of total caloric intake at this age. Mean exposures were 6 mg/kg bw per day for milk- and soy-based formulas (0.03%

carrageenan) and 22 mg/kg bw per day for hydrolysed protein- and/or amino acid-based liquid infant formulas (0.1% carrageenan).

Evaluation

As a general principle, the Committee considers that the ADI is not applicable to infants under the age of 12 weeks, in the absence of specific data to demonstrate safety for this age group.

No studies were available addressing effects of carrageenan on the immature gut, and it was not possible to draw conclusions on whether carrageenan might be absorbed by the immature gut. In addition, there were limited data to indicate whether or not carrageenan can affect the immune response of the gastrointestinal tract, and the nature and potential consequences of such an effect are unknown.

Potential effects of carrageenan in infants could arise from a direct action on the epithelium of the intestinal tract, which would be related to the concentration of carrageenan in infant formula. Alternatively, potential effects could arise from absorption of the low molecular weight fraction of carrageenan, which would be more likely to be related to the dietary exposure expressed on a body weight basis. Therefore, the Committee considered both the concentration of and exposure to carrageenan. The margin of exposure between the concentration in drinking-water reported to cause inflammatory effects in mice and the maximum concentration (0.1%) of carrageenan used in infant formula was 10. On a body weight basis, at this maximum concentration, there was a margin of exposure of about 7 between the lowest doses reported to cause inflammatory responses in rats and mice (1100–1300 mg/kg bw per day) and the estimated exposure to carrageenan from infant formula of 160 mg/kg bw per day prior to weaning. For infants of 12 months of age, there was a margin of exposure of 50 between the lowest-effect doses in rats and mice and the estimated mean exposure to 0.1% carrageenan in infant formula and a margin of exposure of 180 for 0.03% carrageenan, not taking into account possible exposure to carrageenan from other foods. The Committee considered all of these margins of exposure to be insufficient to ensure protection of infants fed infant formula containing carrageenan. The Committee was therefore of the view that it is inadvisable to use carrageenan or processed *Eucheuma* seaweed in infant formula intended for infants up to and including 12 months of age.

The Committee previously concluded that the NOEL of 750 mg/kg bw per day for inflammatory responses in the gastrointestinal tract greatly exceeded the estimated human intake of carrageenan or processed *Eucheuma* seaweed of 30–50 mg/person per day from their use as food additives and therefore allocated a group ADI “not specified”. The new information available to the

Committee did not alter this conclusion. The group ADI "not specified" for the sum of carrageenan and processed *Eucheuma* seaweed was maintained for food additive uses in foods other than infant formula.

The existing specifications for carrageenan and processed *Eucheuma* seaweed were revised. For both carrageenan and processed *Eucheuma* seaweed, the method for residual solvents was updated. For carrageenan, minor modifications were made to the table for infrared absorbance. The Committee also recognized that the lead limit of 2 mg/kg for carrageenan was a typographical error and changed it back to 5 mg/kg, as established at the fifty-seventh meeting of the Committee. Additional minor revisions to the carrageenan and processed *Eucheuma* seaweed specifications monographs were made.

An addendum to the toxicological monograph was prepared.

Recommendation

The Committee noted that the previous dietary exposure estimate for carrageenan was made solely using production poundage and may be outdated. The Committee therefore recommended that a new dietary exposure evaluation, employing specific food type and use level information, be undertaken, ensuring that new uses are adequately taken into consideration.

3.1.4 *Cyclotetraglucose and cyclotetraglucose syrup*

Explanation

Cyclotetraglucose was placed on the agenda at the request of the thirty-eighth meeting of CCFAC under the name cyclotetraose (*7*). The Committee considered that the name cyclotetraose was misleading, as it suggests that the substance is a four-carbon sugar, whereas it is actually a cyclic tetramer of glucose. The Committee therefore assigned it the name cyclotetraglucose. In reaching its decision, the Committee took into account the principles on nomenclature elaborated at its thirty-third meeting (Annex 1, reference *83*). The Committee received information on two types of products, cyclotetraglucose and cyclotetraglucose syrup.

Cyclotetraglucose occurs naturally in sake lees (i.e. the sediment that forms during rice wine production), in sake itself and in the cells of *Saccharomyces cerevisiae*. Cyclotetraglucose is a non-reducing cyclic tetrasaccharide consisting of four D-glucopyranosyl units linked by alternating α(1→3) and α(1→6) glycosidic bonds. The chemical name is *cyclo*[→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→].

Cyclotetraglucose is produced from hydrolysed food-grade starch by the action of a mixture of 6-α-glucosyltransferase (6-GT) and α-isomaltosyltransferase (IMT) derived from *Sporosarcina globispora* and cyclodextrin glycosyltransferase derived from *Bacillus stearothermophilus*. After purification, the product is obtained as either cyclotetraglucose or cyclotetraglucose syrup. Cyclotetraglucose contains not less than 98% cyclotetraglucose, whereas cyclotetraglucose syrup contains 30–40% cyclotetraglucose, both calculated on the anhydrous basis. Cyclotetraglucose and its branched derivatives comprise about 45–55% of cyclotetraglucose syrup. The syrup also contains 15–20% mono-, di- and trisaccharides, as well as about 30% of a variety of unidentified saccharides.

Cyclotetraglucose was placed on the agenda for evaluation as a carrier and stabilizer; however, the manufacturer indicated that cyclotetraglucose and cyclotetraglucose syrup could be used as a dietary fibre. The Committee evaluated cyclotetraglucose for use in food as a carrier for flavours, polyunsaturated fatty acids and vitamins and as a food ingredient. It is stressed that the Committee evaluated the safety of the estimated dietary exposures to cyclotetraglucose resulting from the proposed use levels as a food ingredient only, assuming that these encompassed the much lower levels of use as a carrier and stabilizer. At its sixty-third meeting, the Committee noted that the evaluation of health, nutrient or other claims for food ingredients is outside its remit (Annex 1, reference *173*). Therefore, the Committee did not assess the merit of cyclotetraglucose or cyclotetraglucose syrup as a dietary fibre.

Cyclotetraglucose and cyclotetraglucose syrup have not been previously evaluated by the Committee.

Toxicological data

Studies in animals and humans consistently indicate that cyclotetraglucose largely escapes hydrolysis and absorption in the upper gastrointestinal tract and is only slowly degraded by the intestinal microflora. In vitro, cyclotetraglucose was not hydrolysed by human salivary or porcine pancreatic α-amylase or by artificial gastric juice preparations, and less than 1% incurred ring opening in the presence of rat intestinal mucosa to form a linear saccharide.

The small fraction that may be subject to enzymatic hydrolysis is expected to be absorbed as glucose. However, 12 h following administration of cyclotetraglucose (100 mg/kg bw) to rats via oral gavage, 94% of the administered dose was collected in the faeces, 6% was identified in the gastrointestinal tract and none was detected in blood, indicating that cyclotetraglucose was not metabolized or absorbed in rats. Although no studies on the metabolic fate of cyclotetraglucose in humans were available, the absence

of an increase in plasma glucose and insulin levels following consumption of cyclotetraglucose provides indirect evidence that cyclotetraglucose was not hydrolysed to glucose. Furthermore, cyclotetraglucose was not detected in blood. Conversely, glycaemic and insulinaemic responses were observed in humans ingesting cyclotetraglucose syrup, likely due to the presence of digestible linear carbohydrate components in the syrup.

Following incubation with human stool samples for 24 h, fermentation of cyclotetraglucose was demonstrated to be highly variable, ranging from <5% to 25% within the first 6 h and from <5% to 100% by the end of the 24-h incubation period. A similarly variable fermentation profile was reported for cyclotetraglucose syrup.

A number of acute and short-term toxicity studies were reviewed, which indicated low toxicity by the oral route. No adverse effects were reported in groups of male and female rats given single doses of cyclotetraglucose or cyclotetraglucose syrup at up to 5000 mg/kg bw. Likewise, cyclotetraglucose was not associated with any toxicity in rats following single-dose (2000 mg/kg bw) topical application. The results of 90-day rat toxicity studies with cyclotetraglucose as well as cyclotetraglucose syrup indicated that dietary concentrations of up to 10% (approximately 7000 mg/kg bw) were not associated with any toxicologically significant adverse effects. The results of in vitro genotoxicity assays were negative. No long-term studies of toxicity, reproductive/developmental toxicity or carcinogenicity have been conducted with cyclotetraglucose; however, the Committee concluded that given the known fate of the compound in the gastrointestinal tract, such studies were not required for an evaluation.

Cyclotetraglucose was shown not to possess ocular or dermal irritating or sensitization properties when applied undiluted or moistened with water. Rats given oral cyclotetraglucose doses of up to 2580 mg/kg bw showed increased absorption of minerals (calcium, magnesium, iron and phosphorus). In studies conducted specifically to assess the potential for caecal enlargement as a result of cyclotetraglucose consumption, an effect commonly observed with non-digestible materials, increases in the weight of the caecal contents were observed in both mice and rats following dietary administration of cyclotetraglucose or spray-dried cyclotetraglucose syrup at doses ranging from 5000 to 7500 mg/kg bw. Increases in caecal content weights were accompanied by decreased caecal pH levels and increases in short-chain fatty acid and bile acid levels, effects that are typically encountered with materials that traverse the upper segments of the gastrointestinal tract without digestion, but are fermented in the colon. Additionally, rats maintained on cyclotetraglucose-supplemented diets generally exhibited reduced organ fat content, together with reductions in serum cholesterol and triglyceride levels. These effects were not observed in hamsters.

In a multiple dose level, single-administration human tolerance trial involving 28 adults, a dose-dependent increase was observed in the incidence of gastrointestinal complaints following consumption of single doses of cyclotetraglucose solutions. None of the study subjects experienced laxation after ingestion of 5 or 10 g of the cyclotetraglucose solution; however, at the two higher dose levels evaluated (20 and 30 g), dose-related increases were noted in the occurrence of laxation. In a clinical study involving 40 adults, laxation was reported at dose levels of 40 g of cyclotetraglucose syrup and greater (55 and 70 g), with incidences increasing in proportion to the dose level of the syrup. No laxative effects were reported following consumption of 25 g of cyclotetraglucose syrup. The difference in laxative properties of cyclotetraglucose and cyclotetraglucose syrup is due to the fact that the syrup contains only about 30–40% cyclotetraglucose. No studies involving repeat administration of cyclotetraglucose were provided to the Committee for review.

The 6-GT/IMT enzyme preparation, which is used in the production of cyclotetraglucose and cyclotetraglucose syrup, was reported to be derived from a strain N75 isolated from a soil sample and identified as belonging to *Bacillus globisporus* (currently *Sporosarcina globispora*). No information was provided to support this classification, nor were data provided on the pathogenicity or toxigenicity of the source organism.

Assessment of dietary exposure

The predicted daily dietary exposures to cyclotetraglucose or cyclotetraglucose syrup for consumers only were based on food consumption data derived from individual dietary records reported in the 1994–1998 surveys for the USA and proposed maximum levels of use in a variety of foods as food ingredients, assuming that these levels encompassed the much lower levels of use as a carrier and stabilizer. Dietary exposure estimates from naturally occurring sources, such as sake, were not included in this assessment, as these sources are not commonly consumed by the population in the USA. The contribution from yeast was not considered to be of significance. The mean daily dietary exposure estimate for consumers only in the United States population aged 2 years and above was 12 g/day and for the 90th percentile dietary exposure was 20 g/day (210 and 430 mg/kg bw per day, respectively). The highest dietary exposures were predicted for 13- to 19-year-olds, whose mean and 90th percentile dietary exposures were 12 and 21 g/day, respectively. On a body weight basis, children aged 2–5 years had the highest predicted mean and 90th percentile dietary exposures for cyclotetraglucose or cyclotetraglucose syrup (610 and 980 mg/kg bw per day, respectively). Similar levels of dietary exposure were predicted for Australian and New Zealand populations. The highest predicted dietary exposure to cyclotetraglucose or

cyclotetraglucose syrup at a single eating occasion was 10 g/person and from a single food 14 g/person, both figures for 13- to 19-year-olds in the population in the USA.

Evaluation

Cyclotetraglucose was listed on the agenda for evaluation as a stabilizer and carrier; however, it was brought to the attention of the Committee that cyclotetraglucose may have additional uses as a food ingredient. The Committee concluded that the existing data are adequate to support the safety of cyclotetraglucose and cyclotetraglucose syrup, provided that data are submitted to the Committee regarding the identity of the bacterial strain used to produce the 6-GT/IMT enzyme preparation and evidence of its lack of pathogenicity and toxigenicity. A temporary ADI "not specified" was allocated for cyclotetraglucose and cyclotetraglucose syrup pending submission of these additional data.

Although the highest predicted dietary exposure from a single eating occasion is lower than the laxative dose of approximately 20 g/day, the Committee noted that laxative effects should be taken into account when considering appropriate levels of use of cyclotetraglucose or cyclotetraglucose syrup as food ingredients.

A toxicological monograph was prepared. A Chemical and Technical Assessment and new specifications for cyclotetraglucose and cyclotetraglucose syrup were prepared. The specifications for the syrup were made tentative pending submission of further information on the total saccharide content and test methods and the unidentified saccharide fraction.

The temporary ADI for cyclotetraglucose and cyclotetraglucose syrup and the tentative specifications for cyclotetraglucose syrup will be withdrawn if the requested data are not received by the end of 2008.

3.1.5 *Isoamylase from* Pseudomonas amyloderamosa

Explanation

At the request of CCFAC at its thirty-eighth session (*7*), the Committee evaluated the enzyme isoamylase (glycogen α-1,6-glucanohydrolase; EC 3.2.1.68). The Committee had reviewed toxicological data on this enzyme at its fifty-fifth meeting (Annex 1, reference *149*) as part of the safety assessment of trehalose. Isoamylase catalyses the hydrolysis of 1,6-α-D-glucosidic branch linkages in glycogen, amylopectin and their beta-limit dextrins. It is to be used in the production of food ingredients from starch (e.g. glucose syrup, maltose and maltitol, trehalose, cyclodextrins and resistant starch), typically in combination with other amylolytic enzymes.

Production strain

The production strain is *Pseudomonas amyloderamosa* MU 1174, an isoamylase-hyperproducing strain that was obtained from the wild-type strain SB-15 by means of chemical mutagenesis and selection procedures. The source organism *P. amyloderamosa* is not pathogenic or toxigenic, as evidenced by the results of two acute toxicity studies and one pathogenicity study in mice, and has a history of use in food for more than 20 years.

Chemical and technical considerations

Isoamylase is produced by pure culture fermentation of the production strain *P. amyloderamosa* MU 1174. It is secreted into the fermentation medium, from which it is recovered and concentrated. It is subsequently stabilized, formulated and standardized with maltose, glucose, water and either glycerol fatty acids or sodium benzoate. The isoamylase enzyme preparation conforms to 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*).

The recommended use level of isoamylase in the production of starch-derived food ingredients is up to 5000 isoamylase units/g starch. At the end of the production processes, isoamylase is usually inactivated (typically by heat treatment) or removed.

Toxicological data

Toxicological studies were performed with an isoamylase LEC. The Committee noted that the materials added to the isoamylase LEC for stabilization, formulation and standardization either have been evaluated previously by the Committee or are common food constituents and do not raise safety concerns.

In a 13-week study of toxicity in rats, no significant treatment-related effects were seen when the stabilized LEC was administered by oral gavage at doses up to and including 370 mg TOS/kg bw per day. Therefore, 370 mg TOS/kg bw per day, the highest dose tested, was taken to be the NOEL. The LEC was not mutagenic in an assay for mutagenicity in bacteria in vitro and was not clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro.

Assessment of dietary exposure

Based on a maximum mean daily consumption of 320 g of starch-derived carbohydrates (GEMS/Food Consumption Cluster Diet B) by a 60-kg adult and on the assumptions that the enzyme is used at the maximum recommended use level and that all TOS originating from the enzyme preparation

are carried over into the final products, the dietary exposure would be 0.8 mg TOS/kg bw per day.

Evaluation

Comparing the conservative exposure estimate with the NOEL from the 13-week study of oral toxicity, the margin of safety is approximately 460. The Committee allocated an ADI "not specified" for isoamylase from the production strain *P. amyloderamosa*, 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 *Magnesium sulfate*

Explanation

At the present meeting of the Committee, magnesium sulfate was evaluated in the context of its use as a flavour enhancer, firming agent, fermentation aid and nutrient. Magnesium sulfate occurs in various forms, such as the anhydrous, monohydrate and heptahydrate ($MgSO_4 \cdot 7H_2O$, Epsom salts) forms.

The Committee at its sixty-third meeting evaluated magnesium sulfate and assigned tentative specifications with a request for information on the functional uses of magnesium sulfate other than as a nutrient supplement and their use levels, as well as information on the commercial use of anhydrous magnesium sulfate (Annex 1, reference *173*). A safety evaluation was postponed pending clarification on these issues.

The Committee has previously evaluated other magnesium salts. At the ninth meeting of the Committee (Annex 1, reference *11*), ADIs "not limited" were allocated to magnesium carbonate and magnesium hydroxide.

At the twenty-third meeting of the Committee (Annex 1, reference *50*), ADIs "not specified"[7] were allocated for magnesium chloride, magnesium DL-lactate, magnesium hydrogen carbonate and magnesium gluconate.

At its twenty-ninth meeting (Annex 1, reference *70*), the Committee evaluated magnesium acetate, magnesium adipate, magnesium citrate, magnesium succinate and monomagnesium phosphate and expressed the opinion that ADIs for ionizable salts could be based on the constituent cations and anions. No ADIs were allocated because of the lack of specific information regarding

[7] At its eighteenth meeting (Annex 1, reference *35*), the Committee replaced the term ADI "not limited" with ADI "not specified".

manufacture or use of the food-grade materials. The Committee concluded that the use of magnesium salts as food additives was acceptable provided that the following were taken into consideration:

1. The minimum laxative effective dose is approximately 1000 mg of magnesium moiety from a magnesium salt (observed only when the magnesium salt is administered as a single dose).
2. Infants are particularly sensitive to the sedative effects of magnesium salts.
3. Individuals with chronic renal impairment retain 15–30% of administered magnesium.

At its thirty-first meeting (Annex 1, reference *77*), the Committee allocated an ADI "not specified" to magnesium di-L-glutamate, and at its fifty-first meeting (Annex 1, reference *137*), the Committee reaffirmed the ADI "not specified" for magnesium gluconate.

With respect to the sulfate moiety, at its twenty-ninth meeting (Annex 1, reference *70*), the Committee allocated an ADI "not specified" for potassium sulfate, and at its fifty-seventh meeting (Annex, reference *154*), the Committee allocated an ADI "not specified" for sodium sulfate.

Chemical and technical considerations

Magnesium sulfate is commercially available as a heptahydrate, monohydrate, anhydrous or dried form containing the equivalent of 2–3 waters of hydration. Magnesium sulfate occurs naturally in seawater, in mineral springs and in minerals such as kieserite and epsomite. Magnesium sulfate heptahydrate is manufactured by dissolution of kieserite in water and subsequent crystallization or by sulfation of magnesium oxide. It is produced as a mono- or heptahydrate or in a dried form containing the equivalent of 2–3 waters of hydration.

Magnesium sulfate is used as a nutrient, firming agent and flavour enhancer. It is also used as a fermentation aid in the processing of beer and malt beverages. No food uses have been identified for the anhydrous form of magnesium sulfate.

Assessment of dietary exposure

The only information provided to the present Committee on magnesium sulfate for evaluation was that contained in the submission for the use of magnesium sulfate heptahydrate as an additive and in tabletop salt substitutes (*9*). An independent literature review did not identify any other sources of intake data relating to use of magnesium sulfate in the heptahydrate or other

forms as an additive, although population intakes of magnesium from food and supplements were reported from various national nutrition surveys.

Intakes estimated for magnesium sulfate heptahydrate as an additive were compared with total magnesium intakes from food and supplements and total sulfate intakes from food to place potential intakes of magnesium sulfate from use as an additive in the context of the whole diet (see below).

Intake of magnesium sulfate heptahydrate from its use as an additive. The intake of magnesium sulfate heptahydrate, estimated using production volume data for the USA from 1987, was 31 mg/day, assuming that only 10% of the population were consumers and using a correction factor of 0.6 for under-reporting of the amount of additive produced. This estimated intake is in the same order of magnitude as the more recent and more accurate estimate for the United States population from individual dietary records (Continuing Survey of Food Intakes by Individuals) (*10*). The estimated mean intake of magnesium sulfate heptahydrate for the United States population based on known food uses in beverages only was 13 mg/day, and the 90th percentile intake was 37 mg/day. However, these are likely to be overestimates, as it was assumed that magnesium sulfate heptahydrate was used in all products in each drink category included in the assessment.

Intake of magnesium sulfate from its use as a tabletop salt substitute. The Committee noted that an additional use of magnesium sulfate heptahydrate in tabletop salt substitutes mentioned in the International Council of Beverages Associations (ICBA) submission (*9*) has the potential to increase intake of this additive and magnesium for some people. However, the additional estimated mean intake of magnesium sulfate heptahydrate of 113 mg/day, or 11 mg magnesium/day and 43 mg sulfate/day, from this source is likely to be a gross overestimation, as the estimates submitted to the Committee were derived from data on sodium intakes, where all sodium reported as consumed in the 1999–2000 National Health and Nutrition Examination Survey (*11*) was assumed to be sodium chloride, and all added salt (11% used at table and in cooking) (*12*) was assumed to be replaced by a salt substitute containing magnesium sulfate heptahydrate. In reality, salt substitutes make up a small proportion of tabletop salt use, and not all contain this magnesium salt.

Comparison of intakes of magnesium sulfate heptahydrate from its use as an additive with intakes of magnesium and sulfate from the diet. To put the potential intake of magnesium sulfate into the context of the whole diet, intakes of magnesium and sulfate from known use of magnesium sulfate heptahydrate as an additive in the USA were compared with reported total magnesium intakes (from natural sources in food and use of all food additives containing magnesium) and total sulfate intakes (from sulfur-containing amino acids in food). Mean intakes of magnesium ranged from 220 to 375 mg/day from

national nutrition survey data for selected countries and were of the same order of magnitude as that reported for the United States population (*13*). The intake of magnesium from magnesium sulfate heptahydrate use as a food additive was <4 mg/day (90th percentile intake) and is likely to contribute <2% of the total magnesium intake. Estimated mean sulfate intakes from sulfur-containing amino acids of 1940 mg/day were reported for the United States population only (*12*). The intake of sulfate from magnesium sulfate heptahydrate use as a food additive was <15 mg/day (90th percentile intake) and is likely to contribute <1% of total sulfate intake.

Additional intake of magnesium sulfate from its use in supplements. Nutrient supplements may also contribute to total magnesium intake; however, the ICBA submission indicated that magnesium sulfate was not reported for use in supplements (*14*). Estimates vary as to the potential size of the contribution from all magnesium salts used as supplements to total magnesium intakes, from <2.5% of total magnesium intakes from food for the United Kingdom adult population (*15*) to <40% for the United States population (100 mg magnesium/day from supplements in addition to a mean intake of magnesium of 263 mg/day from food) (*14*). It is noted that for 95th percentile consumers of supplements containing magnesium in the USA, the reported intake of magnesium from this source is at or exceeds the upper level of intake for supplementary magnesium for adults of 350 mg/day established by the USA and adopted in some other countries (*13*, *16*, *17*).

Evaluation

Taking into consideration 1) the widespread occurrence of magnesium and sulfate in the food supply from natural sources and use of other magnesium salts as additives, 2) the uses of magnesium sulfate as a flavour enhancer, firming agent, fermentation aid and nutrient and 3) the previous guidance of the Committee regarding various cation and anion combinations, the Committee allocated an ADI "not specified" for magnesium sulfate.

The use of magnesium sulfate as an additive or in tabletop salt substitutes results in intakes that are well below the reported level of magnesium moiety from a magnesium salt that can cause laxative effects at 1000 mg/day if administered in a single dose (*18*) or the maximum level set in drinking-water quality guidelines of 500 mg/l (e.g. *19*, *20*).

A toxicological monograph was not prepared. A Chemical and Technical Assessment was prepared. The existing specifications were revised, and the tentative designation was removed.

3.1.7 *Phospholipase A1 from* Fusarium venenatum *expressed in* Aspergillus oryzae

Explanation

Phospholipase A1 (phosphatidylcholine 1-acylhydrolase; EC 3.1.1.32) is an enzyme that acts specifically on the fatty acid in position 1 in phospholipid substrates, resulting in the formation of lysophospholipids and free fatty acids. The phospholipase A1 enzyme preparation under evaluation is produced by submerged fermentation of an *Aspergillus oryzae* production strain carrying a gene encoding phospholipase A1 from *Fusarium venenatum* and is to be used in the dairy industry to produce modified phospholipids in milk used for the manufacture of cheese. This enzyme preparation was evaluated previously by the Committee at its sixty-fifth meeting (Annex 1, reference *178*). The Committee at that meeting concluded that the information provided on phospholipase A1 was too limited to allow an assessment of its safety and that the results of two adequate studies of genotoxicity (including a test for chromosomal aberration in mammalian cells in vitro) and a study of toxicity in vivo would be needed. Alternatives to toxicity testing in vivo would be the demonstration that no unintended compounds are present in the enzyme preparation or better molecular characterization of the production strain. At its present meeting, the Committee evaluated new studies on the toxicity in vivo and genotoxicity of phospholipase A1 and re-evaluated the dietary exposure.

Genetic modification / Chemical and technical considerations

The Committee at its sixty-fifth meeting (Annex 1, reference *178*) concluded that the host organism *A. oryzae* is not pathogenic and has a long history of use in food and that the production strain for phospholipase A1, *A. oryzae* PFJo142, constitutes a safe strain lineage. It also concluded that the phospholipase A1 enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing, prepared by the Committee at its fifty-seventh meeting (Annex 1, reference *154*), and that the enzyme preparation is free from the production organism and recombinant DNA.

Toxicological data

Toxicological studies were performed with a phospholipase A1 LEC. The Committee noted that the materials added to the phospholipase A1 LEC for stabilization, formulation and standardization (e.g. glycerol, sucrose, sodium benzoate and potassium sorbate) either have been evaluated previously by the Committee or are common food constituents and do not raise safety concerns.

In a 13-week study of toxicity in rats, no significant treatment-related effects were seen when the LEC was administered by oral gavage at doses up to and including 575 mg TOS/kg bw per day. Therefore, 575 mg TOS/kg bw per day, the highest dose tested, was taken to be the NOEL. The LEC was not mutagenic in an assay for mutagenicity in bacteria in vitro and was not clastogenic in an assay for chromosomal aberrations in mammalian cells in vitro.

Assessment of dietary exposure

Based on a maximum mean daily consumption of 44 g of cheese (GEMS/Food Consumption Cluster Diet E) by a 60-kg adult and on the assumptions that the enzyme is used at the maximum recommended use level of 350 lecitase units/l milk and that all TOS originating from the enzyme preparation remain in the cheese, the dietary exposure would be 0.03 mg TOS/kg bw per day.

Evaluation

Comparing the conservative exposure estimate with the NOEL from the 13-week study of oral toxicity, the margin of safety is >19 000. The Committee allocated an ADI "not specified" for phospholipase A1 from this recombinant strain of *A. oryzae*, used in the applications specified and in accordance with good manufacturing practice.

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

3.1.8 *Sodium iron(III) ethylenediaminetetraacetic acid (sodium iron EDTA)*

Explanation

At the request of CCFAC at its thirty-eighth session (*7*), the Committee re-evaluated the safety of sodium iron(III) ethylenediaminetetraacetic acid trihydrate (in short, sodium iron EDTA) as to its use for iron fortification. The Committee had previously evaluated sodium iron EDTA at its forty-first and fifty-third meetings for its specific application in supervised food fortification programmes in populations in which iron deficiency anaemia is endemic (Annex 1, references *107* and *143*). It was concluded that sodium iron EDTA could be considered safe for use in such programmes, providing daily intakes of iron and EDTA of approximately 0.2 and 1 mg/kg bw, respectively. This opinion has been perceived as being restrictive to the use of sodium iron EDTA as a source of iron for food fortification (i.e. preventing it from being used at levels higher than 0.2 mg iron/kg bw per day and other than "under supervision only"). The Committee at its present meeting was therefore asked to re-evaluate the safety of sodium iron EDTA for iron fortification.

Toxicological data

In addition to the studies already evaluated by the Committee at its earlier meetings, several new studies on the biochemical and toxicological aspects and on the efficacy of sodium iron EDTA were submitted.

The new data provided on the biochemical aspects following administration of sodium iron EDTA corroborate the findings from earlier studies, i.e. that

- the iron in sodium iron EDTA dissociates from the chelate and is released into the common non-haem iron pool before absorption;
- only a very small fraction of the sodium iron EDTA complex (less than 1–2%) is absorbed intact and is rapidly and completely excreted via the kidneys in the urine;
- iron from sodium iron EDTA is generally more efficiently absorbed than iron from alternative sources such as iron(II) sulfate, with less influence of inhibitory factors present in the diet (e.g. phytic acid, polyphenols);
- the same enhancing effect on iron absorption can be achieved by adding disodium EDTA to other soluble iron fortificants, such as iron(II) sulfate.

Moreover, it was demonstrated that the human body maintains iron levels through down-regulating systems, which control the amount of iron absorbed and protect against the possibility of iron overload: more iron is absorbed when the body is in a state of iron deficiency (with an inverse relation between absorption and concentration of serum ferritin) and less when the body iron stores are replete. Besides, a 3-fold increase in the level of iron fortification resulted in a decreased percentage of iron absorption from sodium iron EDTA in healthy adults. Also, in iron-replete subjects, non-haem iron absorption was reduced to a larger extent than the absorption of haem iron, whereas absorption of iron during states of iron depletion was comparable for the two different forms of iron. This tight regulation of non-haem iron absorption is of importance, given that iron from sodium iron EDTA will join the non-haem iron pool before absorption. These findings support the conclusion that dietary iron fortification with sodium iron EDTA does not increase the risk for iron accumulation beyond normal physiological requirements in iron-replete individuals. Studies have also provided additional evidence that dietary intake of sodium iron EDTA has no negative influence on the absorption of other minerals, such as zinc.

From the new toxicological studies provided, it can be concluded that sodium iron EDTA is of low acute oral toxicity and that it does not induce gene mutations in bacteria and mammalian cells in vitro, unless tested at high, cytotoxic concentrations.

The results of new intervention studies in populations with a high prevalence of anaemia and iron deficiency in Viet Nam, China and Kenya demonstrated the efficacy of sodium iron EDTA-fortified condiments (soy sauce and fish sauce) and sodium iron EDTA-fortified whole maize flour in reducing iron deficiency and/or iron deficiency anaemia and the prevalence of anaemia.

Evaluation

The Committee considered the new data submitted to be in support of the earlier conclusions by the Committee at its fifty-third meeting that once the nutritional requirement for iron is satisfied, administration of iron in the form of sodium iron EDTA will not result in greater uptake of iron than from other iron fortificants (such as iron(II) sulfate), as a result of down-regulating systems in the body. The Committee also noted that the new data submitted provide additional evidence that dietary intake of sodium iron EDTA has no adverse effects on the absorption of other minerals, such as zinc.

The Committee concluded that sodium iron EDTA is suitable for use as a source of iron for food fortification to fulfil the nutritional iron requirements, provided that the total intake of iron from all food sources including contaminants does not exceed the provisional monthly tolerable daily intake (PMTDI) of 0.8 mg/kg bw (Annex 1, reference *62*). Additionally, the total intake of EDTA should not exceed acceptable levels, also taking into account the intake of EDTA from the food additive use of other EDTA compounds. An ADI of 0–2.5 mg/kg bw was previously established for the calcium disodium and disodium salts of EDTA, equivalent to up to 1.9 mg EDTA/kg bw (Annex 1, reference *32*). A preliminary exposure assessment based on suggested levels of fortification for sodium iron EDTA indicates that the intake of EDTA in infants and children up to the age of 13 already is at or exceeds the upper limit of the ADI for EDTA.

As previously noted for ferrous glycinate (Annex 1, reference *166*), products, including sodium iron EDTA, that are intended to provide a source of additional iron should not be consumed by individuals with any type of iron storage disease, except under medical supervision.

An addendum to the toxicological monograph was prepared.

3.1.9 *Steviol glycosides*

Explanation

Steviol glycosides are natural constituents of the plant *Stevia rebaudiana* Bertoni. 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 needs for further information. 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 established a temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, on the basis of the NOEL of 970 mg stevioside/kg bw per day (or 383 mg/kg bw, expressed as steviol) in a 2-year study in rats and a safety factor of 200. The total safety factor 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 a need for studies involving repeated exposure of normotensive and hypotensive individuals and insulin-dependent and insulin-independent diabetics to dietary and therapeutic doses.

In order to remove the tentative designation from the specifications, the Committee requested further analytical data on the distribution and concentrations of all component steviol glycosides, including those not identified in these tentative specifications; on the method of analysis for the determination of all component steviol glycosides, including those not identified in the tentative specifications; on the nature and concentration of the non-steviol glycoside fractions; on the quantities of residual solvents from purification steps of the manufacturing process; and on the hydrolytic stability of the steviol glycosides in acidic foods and beverages.

At the current meeting, the Committee considered the information that had become available since the sixty-third meeting. This comprised two toxicological submissions, which included a summary of published studies and some unpublished data, additional information identified from the scientific literature and responses intended to resolve the outstanding issues relevant to the specifications. Additionally, the Committee received a request to remove the requirement for a minimum content of the sum of stevioside and rebaudioside A from the specifications. It was noted that such a limit was unnecessary because of the requirement that total steviol glycosides be not less than 95% and because all the steviol glycosides decompose upon ingestion to steviol, on which the temporary ADI was based. The Committee was also informed that results of an ongoing toxicity testing programme, including clinical studies, would be available by August 2007.

Chemical and technical considerations

The Committee received more detail on the process of purification of the additive to support the specified high level of purity.

The Committee examined results of thermal and hydrolytic stability studies for the material under evaluation. Isosteviol, glucose and "oligoglucose" were identified as the only decomposition products of steviol glycosides that had been subjected to various conditions of pH and temperature.

A summary of literature studies that addressed the stabilities of stevioside and rebaudioside A was available to the Committee. Although the summarized studies contained no information on the purities of these two substances, the Committee found the information helpful for the present evaluation because the specified material includes products that may be 95% stevioside or 95% rebaudioside A.

Toxicological data

A number of studies provide further information on the metabolism of steviol glycosides in humans, which will support future risk assessments.

The newly published data mainly involved studies of effects of steviol glycosides in a range of in vitro and animal models related to diabetes. Although these studies provide additional information on the mechanism of action of steviol glycosides, they did not directly address the Committee's stated requirements.

One new study was available relating to genotoxicity. A stevioside product (purity 88.6%) was administered in drinking-water to rats for 45 days (equivalent to about 200 mg/kg bw per day, expressed as steviol). Increased DNA damage (assessed by a comet assay) was observed in nucleated cells of peripheral blood after 5 and 6 weeks compared with concurrent controls. There were no significant effects in blood cells at earlier time points. Increased DNA damage was seen in liver, brain and spleen cells at termination of the study (*21*). The DNA damage in blood cells of control animals was also increased at weeks 5 and 6 compared with earlier time points, and no positive controls were included. Taking into account the lack of genotoxicity of stevioside in studies reviewed previously and that the product tested in this non-standard assay did not meet the proposed specification for steviol glycosides, the Committee considered that the results of this study were not convincing evidence of genotoxicity.

Three new controlled oral studies of effects of steviol glycosides in humans were available.

In an unpublished study submitted to the Committee, 250 mg of a product containing 91.7% total steviol glycosides, including 64.5% stevioside and 18.9% rebaudioside A, were administered to groups of type 1 ($n = 8$) and type 2 diabetics ($n = 15$) and non-diabetics ($n = 15$) 3 times daily for 3 months in a double-blind, placebo-controlled trial. Control groups with the same number of subjects received a placebo. After 3 months, there were no significant changes in systolic or diastolic blood pressure, glycated haemoglobin, blood lipids or renal or hepatic function. No side-effects were reported (*22*). The Committee noted that this product did not meet the proposed specification of "not less than 95% steviol glycosides" and that the study was conducted in a small number of subjects.

A study of antihypertensive effects was conducted in previously untreated mild hypertensive patients with crude stevioside obtained from the leaves of *S. rebaudiana.* Patients with essential hypertension were subjected to a placebo phase for 4 weeks and then received either capsules containing placebo for 24 weeks or crude stevioside at consecutive doses of 3.75 mg/kg bw per day (7 weeks), 7.5 mg/kg bw per day (11 weeks) and 15 mg/kg bw per day (6 weeks). Comparison of patients receiving stevioside with those on placebo showed neither antihypertensive nor adverse effects of stevioside (*23*). The product in this study also did not meet the proposed specification.

According to a study available in abstract form only, a randomized double-blind, placebo-controlled study was conducted in subjects with type 2 diabetes. Fifty-five subjects received 500 mg stevioside (purity unspecified) or placebo (maize starch) 3 times daily for 3 months. Compared with the placebo, stevioside did not reduce the incremental area under the glucose response curve and maintained the insulin response and glycated haemoglobin and fasting blood glucose levels. No difference in lipids or blood pressure was observed (*24*).

In addition, a study of skin prick allergy testing with 10% stevioside conducted in infants (50 per group, aged 4 months to 2 years) indicated a higher prevalence of sensitization to stevioside in infants with allergic diseases compared with healthy infants (*25*).

Evaluation

The Committee considered that the newly available data did not raise additional concerns regarding the safety of steviol glycosides, but that the results of ongoing clinical studies, which more closely address the requirements specified at the sixty-third meeting, would be essential to its evaluation. The Committee therefore agreed to extend 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. No toxicological monograph was prepared.

The Committee concluded that steviol glycosides are sufficiently thermally and hydrolytically stable for use in foods, including acidic beverages, under normal conditions of processing and storage. The other outstanding issues on method of manufacture and specifications were also adequately resolved.

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, and the Chemical and Technical Assessment prepared by the Committee at its sixty-third meeting was updated.

3.2 Revision of specifications

3.2.1 *Maltol and ethyl maltol*

Maltol and ethyl maltol were evaluated as flavouring agents at the sixty-fifth meeting of the Committee (Annex 1, reference *178*) and given specifications in the flavouring agent format under Nos 1480 and 1481. However, both substances also have specifications in the traditional food additives format, and these were revised at the sixty-fifth meeting and made "tentative" pending the receipt of further information on the functional uses of the two substances and information on the method of assay.

At the present meeting, the necessary revisions were made to the traditional food additives format specifications by deleting the term "stabilizer" from the functional uses of both substances and by including a suitable method of assay in the ethyl maltol specifications. Further revisions were also made to bring the specifications up to date and to align them with the flavouring agent format specifications, which were also amended as needed. The Committee also removed the "tentative" designations from the food additives format specifications for maltol and ethyl maltol.

The overall result was that the traditional food additives format specifications for maltol and ethyl maltol were revised and the "tentative" designations removed, and the flavouring agent format specifications for the two substances under Nos 1480 and 1481 were revised.

3.2.2 *Nisin preparation*

The Committee received a request to revise the existing nisin specifications to acknowledge the use of non-milk-based substances, such as carbohydrate solids and yeast extract, in addition to milk solids in the fermentation

medium. The Committee first evaluated nisin at its twelfth meeting (Annex 1, reference *17*). At the present meeting, specifications were revised by changing the name of the monograph to "nisin preparation", which better represents the commercial product. In addition, the name of the source microorganism and the method of assay were updated, and the non-milk-based substances were included as components of the fermentation medium. Microbiological criteria were added.

3.2.3 *Pectins*

At the request of the industry, the Committee reviewed the method for residual solvents published in the pectins specifications monograph. The Committee agreed to modify the pectins monograph, referring to the residual solvents method published in Volume 4 of the *Combined compendium of food additive specifications* (Annex 1, reference *180*) with a modified version of the sample preparation before analysis.

3.2.4 *Polyvinyl alcohol*

The Committee noted an error in the equation for the calculation of the Acid Value in the specifications monograph for polyvinyl alcohol. The existing specifications were revised to correct the error, which also required amending the analytical procedure for the determination of the Acid Value.

3.2.5 *Sucrose esters of fatty acids*

The Committee noted at its sixty-fifth meeting (Annex 1, reference *178*) that the specifications for sucrose esters of fatty acids referred to a toxic solvent and outdated packed gas chromatography (GC) columns in several analytical procedures. The Committee changed the specifications to tentative and requested further information to update the analytical procedures. At its current meeting, the Committee decided that the information received on the analytical procedures for free sucrose, dimethylformamide, dimethyl sulfoxide, propylene glycol and method of assay was sufficient to remove the tentative designation. Additionally, the Committee noted that the test method for dimethyl sulfoxide still used an outdated GC column and recommended that further efforts be taken to find a suitable replacement.

4. Flavouring agents

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

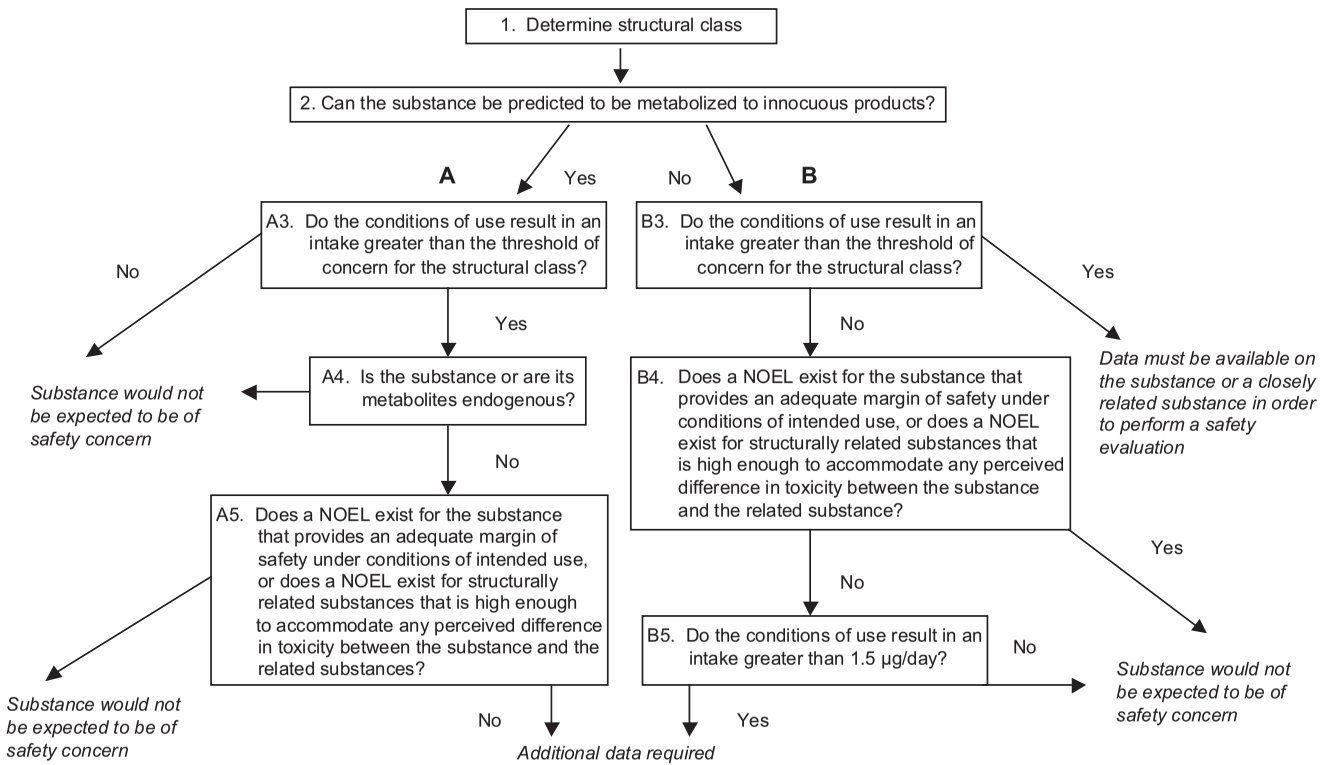
Eight groups of flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents as outlined in Figure 1 (Annex 1, references *116*, *122*, *131*, *137*, *143*, *149*, *154*, *160*, *166*, *173* and *178*). In applying the Procedure, the chemical is first assigned to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

- C*lass 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.
- C*lass 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.
- C*lass 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:

- *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 National Research Council of the National Academy of Sciences (under contract to the Food and Drug Administration) 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 (*3*, *4*, *5*) (see also section 2.5.1).

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

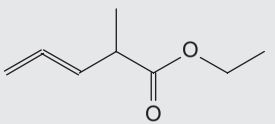
The population of consumers was assumed to be 32×10^6 in Europe, 13×10^6 in Japan and 28×10^6 in the USA.

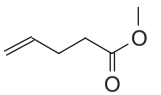
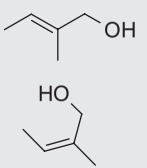
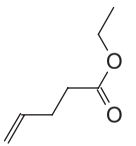
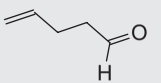
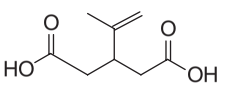
4.1.1 *Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters: additional compounds*

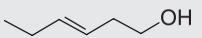
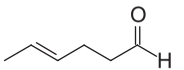
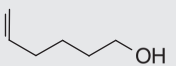
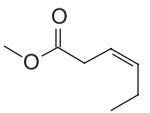
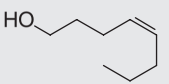
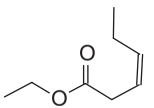
The Committee evaluated a group of 28 flavouring agents, 27 new and 1 previously evaluated (Annex 1, reference *137*), that included linear and branched-chain aliphatic, unsaturated, unconjugated alcohols (6), aldehydes (6), acids (5) and related esters (11) (see Table 2). The evaluations were conducted using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*).

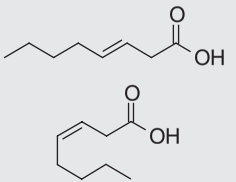
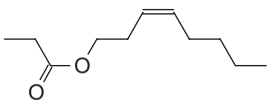
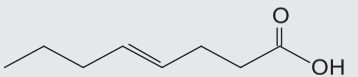
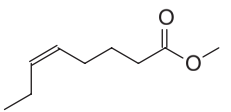
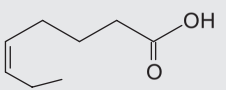
Table 2

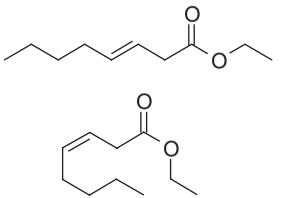
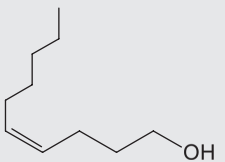
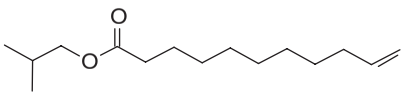
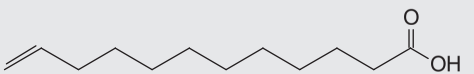
Summary of the results of safety evaluations of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters used as flavouring agents[a, b, c]

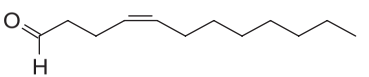
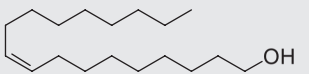
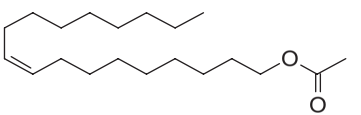
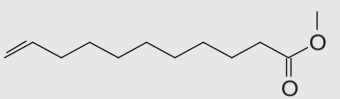
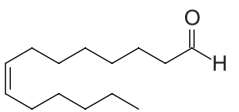
Flavouring agent	No.	CAS No. and structure	*Step A3/B3*[d] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related substances?	Comments on predicted metabolism	Conclusion based on current intake
Ethyl-2-methyl-3,4-pentadienoate	353	60523-21-9 	No Europe: ND USA: 0.01 Japan: 0.03	Yes. The NOEL of 1 mg/kg bw per day from a 92-day study in rats (*26*) is at least 2 million times the estimated daily intake level of ethyl-2-methyl-3,4-pentadienoate when used as a flavouring agent.	See note 1	No safety concern

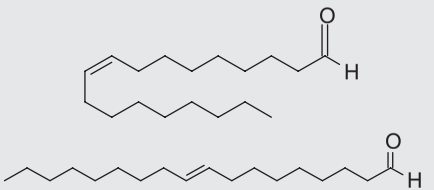
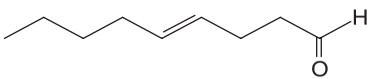
Methyl 4-pentenoate	1616	818-57-5 	No Europe: ND USA: ND Japan: 0.03	NR	See notes 1 and 2	No safety concern
2-Methylbut-2-en-1-ol	1617	4675-87-0 	No Europe: 0.01 USA: ND Japan: ND	NR	See notes 2 and 4	No safety concern
Ethyl 4-pentenoate	1618	1968-40-7 	No Europe: ND USA: ND Japan: 0.07	NR	See notes 1 and 2	No safety concern
4-Pentenal	1619	2100-17-6 	No Europe: 0.1 USA: ND Japan: ND	NR	See note 2	No safety concern
3-Isopropenylpentanedioic acid	1620	6839-75-4 	No Europe: ND USA: ND Japan: 0.03	NR	See note 3	No safety concern

trans-3-Hexenol	1621	928-97-2 	No Europe: 340 USA: 602 Japan: 10	NR	See note 2	No safety concern
trans-4-Hexenal	1622	25166-87-4 	No Europe: 0.01 USA: 0.1 Japan: 0.03	NR	See note 2	No safety concern
5-Hexenol	1623	821-41-0 	No Europe: ND USA: ND Japan: 0.03	NR	See note 2	No safety concern
Methyl (*Z*)-3-hexenoate	1624	13894-62-7 	No Europe: 0.01 USA: 0.2 Japan: ND	NR	See notes 1 and 2	No safety concern
cis-4-Octenol	1625	54393-36-1 	No Europe: ND USA: ND Japan: 0.03	NR	See note 2	No safety concern
Ethyl (*Z*)-3-hexenoate	1626	64187-83-3 	No Europe: 0.01 USA: 0.1 Japan: ND	NR	See notes 1 and 2	No safety concern

3-Octenoic acid	1627	1577-19-1 	No Europe: ND USA: ND Japan: 0.03	NR	See note 3	No safety concern
(*Z*)-3-Octenyl propionate	1628	94134-03-9 	No Europe: 0.01 USA: 0.7 Japan: ND	NR	See notes 1 and 2	No safety concern
trans-4-Octenoic acid	1629	18776-92-6 	No Europe: ND USA: ND Japan: 0.05	NR	See note 3	No safety concern
Methyl (*Z*)-5-octenoate	1630	41654-15-3 	No Europe: 0.01 USA: 0.9 Japan: ND	NR	See notes 1 and 2	No safety concern
cis-5-Octenoic acid	1631	41653-97-8 	No Europe: ND USA: ND Japan: 0.4	NR	See note 3	No safety concern

Ethyl 3-octenoate	1632	1117-65-3 	No Europe: 0.02 USA: ND Japan: 0.03	NR	See notes 1 and 2	No safety concern
cis-4-Decenol	1633	57074-37-0 	No Europe: ND USA: ND Japan: 0.03	NR	See note 2	No safety concern
Isobutyl 10-undecenoate	1634	5421-27-2 	No Europe: ND USA: ND Japan: 0.7	NR	See notes 1 and 2	No safety concern
11-Dodecenoic acid	1635	65423-25-8 	No Europe: ND USA: ND Japan: 9	NR	See note 3	No safety concern

(*Z*)-4-Dodecenal	1636	21944-98-9 	No Europe: 0.01 USA: ND Japan: ND	NR	See note 2	No safety concern
cis-9-Octadecenol	1637	143-28-2 	No Europe: ND USA: ND Japan: 6	NR	See note 3	No safety concern
cis-9-Octadecenyl acetate	1638	693-80-1 	No Europe: ND USA: ND Japan: 6	NR	See notes 1 and 2	No safety concern
Methyl 10-undecenoate	1639	111-81-9 	No Europe: 0.01 USA: 1 Japan: 0.03	NR	See notes 1 and 2	No safety concern
(*Z*)-8-Tetradecenal	1640	169054-69-7 	No Europe: 0.01 USA: ND Japan: ND	NR	See note 2	No safety concern

9-Octadecenal	1641	5090-41-5		No Europe: 0.01 USA: 1 Japan: ND	NR	See note 2	No safety concern
(*E*)-4-Nonenal	1642	2277-16-9		No Europe: ND USA: 0.1 Japan: ND	NR	See note 2	No safety concern

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.

[a] Sixty-two flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *137* and *166*).

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

[c] *Step 2*: All of the flavouring agents in this group except ethyl-2-methyl-3,4-pentadienoate (No. 353) can be predicted 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 to Table 2:

1. Aliphatic unsaturated esters are expected to undergo hydrolysis to form the corresponding alcohol and carboxylic acid. For ethyl-2-methyl-3,4-pentadienoate (No. 353), however, other routes of metabolism are possible.
2. Aliphatic unsaturated alcohols are expected to undergo oxidation to the corresponding aldehyde and carboxylic acid and be completely metabolized in the fatty acid and the citric acid pathways.
3. Anticipated to undergo complete metabolism in the β-oxidation pathway.
4. Expected to be oxidized to an α,β-unsaturated aldehyde prior to the formation of the carboxylic acid. While an α,β-unsaturated carbonyl group is a potential structural alert for toxicity, the protective processes in cells provide adequate capacity for detoxication at the low levels of use as a flavouring agent (Annex 1, reference *154*, section 2.5).

The Committee previously evaluated 42 other members of this chemical group of flavouring agents at its fifty-first meeting (Annex 1, reference *137*). The findings presented in that report were considered in the present evaluation. For 41 of the 42 substances in this group, it was concluded that there were no safety concerns at the currently estimated levels of intake. The evaluation of one substance, ethyl 2-methyl-3,4-pentadienoate (No. 353), was deferred pending review of a 92-day dietary study (*26*). This study has now been provided, and the Committee has evaluated this substance in addition to the other 27 new flavouring agents being evaluated.

The Committee previously evaluated 20 additional flavouring agents from this chemical group at the sixty-first meeting (Annex 1, reference *166*). It was concluded that the 20 substances in this group were of no safety concern at the currently estimated levels of intake.

Ten of the 28 flavouring agents (Nos 1617, 1619, 1621, 1623, 1624, 1626, 1632, 1633, 1637 and 1641) in this group are natural components of foods. These flavouring agents have been detected in fruits and berries; wine, brandy and scotch whiskey; black, rooibos and green teas; coffee; herbs; spearmint oil, hop oil, mastic gum leaf oil, buchu oil and corn oil; honey; leeks and peas; and pork, beef and chicken (*27*). For No. 1641, there were sufficient quantitative data to determine a consumption ratio (the ratio of its consumption from natural food sources to its use as a flavouring agent) of 1416.

Assessment of dietary exposure

The total annual volume of production of these 28 linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters is approximately 3200 kg in Europe, 5000 kg in the USA and 120 kg in Japan (*3*, *4*, *5*).

Greater than 99% of the annual production volume in Europe and the USA is accounted for by *trans*-3-hexenol (No. 1621). The daily per capita intake of *trans*-3-hexenol is 602 µg in the USA, 340 µg in Europe and 10 µg in Japan. Over 95% of the annual production volume in Japan is accounted for by *trans*-3-hexenol, 11-dodecenoic acid (No. 1635), *cis*-9-octadecenol (No. 1637) and *cis*-9-octadecenyl acetate (No. 1638). The daily per capita intake of each flavouring agent is reported in Table 2. The annual volume of production of each flavouring agent is reported in Table 3.

Table 3
Annual volumes of production of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids 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
Ethyl 2-methyl-3,4-pentadienoate (353)			
Europe	ND	NA	NA
USA	0.1	0.01	0.0002
Japan	0.1	0.03	0.0005
Methyl-4-pentenoate (1616)			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.1	0.03	0.0005
2-Methylbut-2-en-1-ol (1617)			
Europe	0.1	0.01	0.0002
USA	ND	NA	NA
Japan	ND	NA	NA
Ethyl 4-pentenoate (1618)			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.3	0.07	0.0012
4-Pentenal (1619)			
Europe	0.9	0.10	0.0016
USA	ND	NA	NA
Japan	ND	NA	NA
3-Isopropenylpentanedioic acid (1620)			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.1	0.03	0.0004
***trans*-3-Hexenol (1621)**			
Europe	3175	340	5.7
USA	4925	602	10.0
Japan	38	10	0.17
***trans*-4-Hexenal (1622)**			
Europe	0.1	0.01	0.0002
USA	1	0.12	0.0020
Japan	0.1	0.03	0.0004
5-Hexenol (1623)			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.1	0.03	0.0004

Methyl (*Z*)-3-hexenoate (1624)			
Europe	0.1	0.01	0.0002
USA	1	0.16	0.0027
Japan	ND	NA	NA
***cis*-4-Octenol (1625)**			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.1	0.03	0.0004
Ethyl (*Z*)-3-hexenoate (1626)			
Europe	0.1	0.01	0.0002
USA	1	0.13	0.0022
Japan	ND	NA	NA
3-Octenoic acid (1627)			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.1	0.03	0.0004
(*Z*)-3-Octenyl propionate (1628)			
Europe	0.1	0.01	0.0002
USA	6	0.73	0.0122
Japan	ND	NA	NA
***trans*-4-Octenoic acid (1629)**			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.2	0.05	0.0008
Methyl (*Z*)-5-octenoate (1630)			
Europe	0.1	0.01	0.0002
USA	7	0.86	0.014
Japan	ND	NA	NA
***cis*-5-Octenoic acid (1631)**			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	1	0.37	0.006
Ethyl 3-octenoate (1632)			
Europe	0.2	0.02	0.0003
USA	ND	NA	NA
Japan	0.1	0.03	0.0004
***cis*-4-Decenol (1633)**			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	0.1	0.03	0.0004
Isobutyl 10-undecenoate (1634)			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	3	0.71	0.012
11-Dodecenoic acid (1635)			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	35	9.14	0.15

(*Z*)-4-Dodecenal (1636)			
Europe	0.1	0.01	0.0002
USA	ND	NA	NA
Japan	ND	NA	NA
***cis*-9-Octadecenol (1637)**			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	21	5.50	0.09
***cis*-9-Octadecenyl acetate (1638)**			
Europe	ND	NA	NA
USA	ND	NA	NA
Japan	25	6.46	0.11
Methyl 10-undecenoate (1639)			
Europe	0.1	0.01	0.0002
USA	8	1.01	0.017
Japan	0.1	0.03	0.0004
(*Z*)-8-Tetradecenal (1640)			
Europe	0.1	0.01	0.0002
USA	ND	NA	NA
Japan	ND	NA	NA
9-Octadecenal (1641)			
Europe	0.1	0.01	0.0002
USA	10	1.22	0.020
Japan	ND	NA	NA
(*E*)-4-Nonenal (1642)			
Europe	ND	NA	NA
USA	1	0.12	0.0020
Japan	ND	NA	NA
Total			
Europe	3177		
USA	4950		
Japan	124		

ND, no data reported; NA, not applicable.

[a] From references *3*, *4*, *5*. Total volume of production values of <1 kg reported in the surveys (*3*, *4*, *5*) have been truncated to one place following the decimal point (0.1 kg).

[b] Intake (μg/person per day) calculated as follows:
[(annual volume of production, 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; where correction factor = 0.8 for the surveys by FEMA USA, Europe and Japan, representing the assumption that only 80% of the annual flavour volume of production was reported in the surveys (*3*, *4*, *5*).
Intake (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur as a result of rounding.

Absorption, distribution, metabolism and elimination

Ten of the 11 linear aliphatic esters (Nos 1616, 1618, 1624, 1626, 1628, 1630, 1632, 1634, 1638 and 1639) in this group are expected to be hydrolysed by digestive fluids to the corresponding aliphatic alcohol and carboxylic acid

(*28*, *29*, *30*, *31*, *32*, *33*). The linear carboxylic acids participate in fatty acid metabolism and are cleaved to yield acetyl or propionyl coenzyme A (CoA), which is metabolized to carbon dioxide and water in the tricarboxylic acid cycle. There is no information available on the metabolism of the branched-chain diene, 2-methyl-3,4-pentadienoate (No. 353).

The linear primary alcohols, once formed, are rapidly absorbed from the gastrointestinal tract and oxidized to their corresponding aldehydes (*34*, *35*), which are, in turn, oxidized to their corresponding carboxylic acids and undergo normal fatty acid metabolism. Minor metabolic pathways, such as ω-oxidation in the liver or α-oxidation in the brain, may also occur.

The four long-chain (C>8) aldehydes (Nos 1636, 1640, 1641 and 1642) in this group are readily absorbed as micelles, oxidized, esterified with glycerol, deposited in chylomicrons or low-density lipoproteins and transported to the liver via the lymphatic system (*36*). Once absorbed, these and the two short-chain aldehydes (Nos 1619 and 1622) are oxidized to their corresponding unsaturated carboxylic acids and undergo normal fatty acid metabolism.

The six unsaturated alcohols (Nos 1617, 1621, 1623, 1625, 1633 and 1637) in this group are expected to be oxidized to the corresponding unsaturated carboxylic acids and, together with the other five unsaturated carboxylic acids (Nos 1620, 1627, 1629, 1631 and 1635) in this group, are expected to undergo normal fatty acid metabolism. Prior to entering the fatty acid pathway, the *cis* isomers within the unsaturated carboxylic acids are converted to *trans* isomers by the action of 3-hydroxyacyl CoA epimerase. Also, in some cases, the double bond is isomerized from the 3- to the 2-position by enoyl CoA isomerase (*37*). 2-Methylbut-2-en-1-ol (No. 1617) would be oxidized to an α,β-unsaturated aldehyde prior to the formation of the carboxylic acid. Flavouring agents that contain an α,β-unsaturated carbonyl group have been previously considered by the Committee (Annex 1, references *131*, *149* and *154*).

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 all 28 flavouring agents (Nos 353 and 1616–1642) to structural class I (*38*).

Step 2. Twenty-seven of the 28 flavouring agents in this group are expected to be metabolized to innocuous products, including 2-methylbut-2-en-1-ol (No. 1617). While this substance is metabolized to an α,β-unsaturated carbonyl group, which is a potential structural alert for toxicity, the protective processes in cells provide adequate capacity for detoxication at the low levels

of use as a flavouring agent (Annex 1, reference *154*, section 2.5). The evaluation of these 27 flavouring agents therefore proceeded via the A-side of the Procedure.

The Committee considered that inadequate data were available to predict the metabolism of ethyl-2-methyl-3,4-pentadienoate (No. 353), because of its terminal diene. The evaluation of this flavouring agent therefore proceeded via the B-side of the Procedure.

Step A3. The estimated daily per capita intakes of the 27 flavouring agents in structural class I that proceeded via the A-side of the Procedure are below the threshold of concern (i.e. 1800 µg/person per day for class I). The Committee concluded that when evaluated according to the Procedure, these 27 flavouring agents do not raise safety concerns when they are used at their currently estimated levels of intake.

Step B3. The estimated daily per capita intake for ethyl-2-methyl-3, 4-pentadienoate (No. 353) is below the threshold of concern (i.e. 1800 µg/person per day). Accordingly, the evaluation of this flavouring agent proceeded to step B4.

Step B4. For ethyl-2-methyl-3,4-pentadienoate (No. 353), the NOEL of 1 mg/kg bw per day from a 92-day study in rats (*26*) provides an adequate margin of safety (at least 2 million) in relation to the estimated levels of exposure from its use as a flavouring agent in the USA (0.0002 µg/kg bw per day) and in Japan (0.0005 µg/kg bw per day).

Table 2 summarizes the evaluations of 28 linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters (Nos 353 and 1616–1642) in this group.

Additional toxicity data

In an acute toxicity study on 4-pentenal (No. 1619), the oral median lethal dose (LD_{50}) in rats was 620 mg/kg bw (*39*).

In a short-term toxicity study with ethyl-2-methyl-3,4-pentadienoate (No. 353), a group of 15 male and 15 female Sprague-Dawley rats was administered ethyl 2-methyl-3,4-pentadienoate mixed in a basal diet at a level equivalent to a daily intake of 0.95 mg/kg bw per day for males and 1.0 mg/kg bw per day for females for 92 days. An additional group of animals was provided an unmodified basal diet and served as the control. Daily observation of animals revealed scattered signs of bloody nasal discharge, dry blood around the nose and rales; however, these effects were observed in both the test group and the controls. Weekly measurements of body weights and food consumption revealed significant decreases ($P < 0.05$) in both parameters in

test females compared with controls. Efficiency of food utilization in test females was unchanged, except during week 2, when it decreased, and during week 9, when it increased. In males, a significant decrease in food consumption extending over the entire study period and a significant increase in the efficiency of food utilization during week 8 were reported; however, there was no biological significance to these findings. Haematological examinations, clinical chemistry and urinary analysis performed at the end of weeks 6 and 12 (eight animals per sex per group) revealed no differences between treated and control groups. At necropsy, no differences were observed in absolute or relative organ weights (i.e. liver, spleen, kidneys, adrenals and thyroid) between test and control animals. Macroscopic and histopathological examinations did not reveal any treatment-related effects. The NOEL was 1 mg/kg bw per day (*26*).

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are predicted to be metabolized by hydrolysis and/or oxidative metabolism of alcohol and aldehyde groups and alkyl side-chains. 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 at this meeting and at the fifty-first and sixty-first meetings are predicted to be metabolized to common metabolites. Common metabolites (and their precursors) are 4-pentenoic acid (Nos 314, 1270, 1616, 1618 and 1619), 3-hexenoic acid (Nos 315, 316, 317, 334, 335, 336, 1271, 1272, 1274, 1275, 1276, 1277, 1278, 1279, 1621, 1624 and 1626), 4-hexenoic acid (Nos 318, 319 and 1622), 5-hexenoic acid (Nos 1273 and 1623), 4-heptenoic acid (Nos 320, 1280 and 1281), 3-octenoic acid (Nos 321, 1627, 1628 and 1632), 4-octenoic acid (Nos 337, 338, 1625 and 1629), 5-octenoic acid (Nos 322, 323, 1282, 1630 and 1631), 4-nonenoic acid (No. 1642), 6-nonenoic acid (Nos 324 and 325), 4-decenoic acid (Nos 326, 341, 1287, 1288 and 1633), 10-undecenoic acid (Nos 330, 331, 343, 344, 1634 and 1639) and 9-octadecenoic acid (Nos 1637, 1638 and 1641). The combined intakes of substances with a common metabolite were below the threshold for class I, except for those substances metabolized to 3-hexenoic acid. In this case, the combined intake did not add significantly to the intake of *cis*-3-hexen-1-ol (No. 315), which exceeded the class I threshold and was evaluated at the fifty-first meeting and considered not to be a safety concern. The Committee concluded that under the conditions of use as flavouring agents, the combined intake 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

Six members of this group of flavouring agents, *trans*-4-hexenal (No. 1622), (*Z*)-4-dodecenal (No. 1636), *cis*-9-octadecenol (No. 1637), *cis*-9-octadecenyl acetate (No. 1638), 9-octadecenal (No. 1641) and (*E*)-4-nonenal (No. 1642), have assay values of <95%. Information on the safety of the secondary components of these six 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 in No. 1622 (*cis*-4-hexenal [No. 319], 3-hexen-1-ol [No. 315] and hexanal [No. 92]) are expected to share the same metabolic fate as *trans*-4-hexenal and are considered not to present a safety concern at current levels of intake. The secondary component of No. 1636 (dodecanal) is expected to share the same metabolic fate as (*Z*)-4-dodecenal. The secondary components of No. 1637 (octadecanol and hexadecanol) are expected to share the same metabolic fate as *cis*-9-octadecenol. The secondary components of No. 1638 (hexadecyl acetate and octadecyl acetate) are expected to share the same metabolic fate as *cis*-9-octadecenyl acetate. The secondary component of No. 1641 (octadecenal) is expected to share the same metabolic fate as 9-octadecenal. The secondary components of No. 1642 (2-nonen-4-ol and 2*E*,4*E*-nonadienal) are expected to share the same metabolic fate as (*E*)-4-nonenal.

Conclusion

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

The Committee concluded that these 28 flavouring agents, which are additions to the two groups of linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters evaluated previously, do not raise any safety concerns at the currently estimated levels of intake.

No toxicological monograph was prepared.

4.1.2 *Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances: additional compounds*

The Committee evaluated a group of 15 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances that included 3 terpene alcohols, 3 aliphatic tertiary alcohols, 3 phenyl-substituted aliphatic

alcohols, 4 esters of phenyl-substituted aliphatic alcohols and 2 spiranes. The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these agents has previously been evaluated.

The Committee previously evaluated 23 other members of this chemical group of flavouring agents at its fifty-first meeting (Annex 1, reference *137*). The findings presented in that report were considered in the present evaluation. Twenty-two of the 23 substances evaluated at the fifty-first meeting were concluded to be of no safety concern based on currently estimated levels of intake. The Committee concluded that additional data were required for the evaluation of methyl 1-acetoxycyclohexylketone (No. 442).

Five of the 15 flavouring agents evaluated in the current group are natural components of foods (Nos 1646 and 1650–1653). They have been detected in a wide variety of foods, including pepper, orange juice and peel, lemon juice, grapefruit juice, berries, pineapple, guava, melon, tomato, mango, beer, sage, parsley, lemon balm, beans, rice, ginger, cocoa, black and green teas, red and white wines, brandy, mango, peppermint oil, spearmint oil, skim milk powder, a variety of other herbs and spices and a number of citrus oils and honeys.

Assessment of dietary exposure

The total annual volume of production of this group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances is approximately 1800 kg in Europe, 13 000 kg in the USA and 960 kg in Japan. Greater than 90% of the annual production volume in Europe, the USA and Japan is accounted for by α,α-dimethylphenethyl acetate (No. 1655) and α,α-dimethylphenethyl butyrate (No. 1656). The daily per capita intake of each agent is reported in Table 4.

Absorption, distribution, metabolism and elimination

It is anticipated that the esters in this group would be readily hydrolysed to their component alcohols and carboxylic acids. The hydrolysis products would be readily metabolized primarily by conjugation with glucuronic acid and are excreted primarily in the urine. Alternatively, alcohols with unsaturation may be ω-oxidized at the allylic position to yield polar metabolites, which may be conjugated and excreted. Metabolites of acyclic alcohols may be further oxidized to eventually yield carbon dioxide.

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 all but two flavouring agents to structural class I. 6-Acetoxydihydrotheaspirane (No. 1647) and 6-hydroxydihydrotheaspirane (No. 1648) were assigned to structural class II.

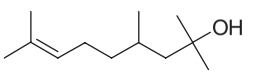
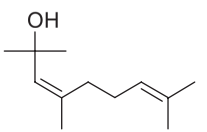
Step 2. All flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all the substances in this group therefore proceeded via the A-side of the Procedure.

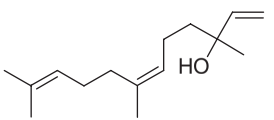
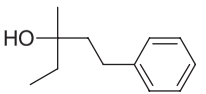
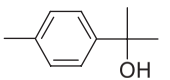
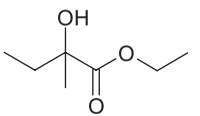
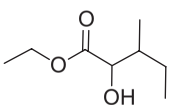
Step A3. The estimated daily per capita intakes for the 13 flavouring agents in structural class I are below the threshold of concern (i.e. 1800 µg/person per day for class I). The estimated daily per capita intakes for the 2 flavouring agents in structural class II are below the threshold of concern (540 µg/person per day for class II). According to the Procedure, the safety of these 15 flavouring agents raises no concern when they are used at their current estimated levels of intake.

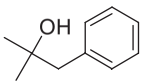
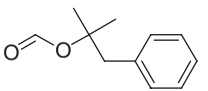
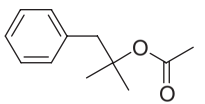
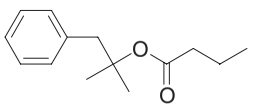
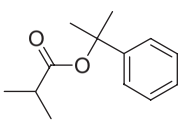
Table 4 summarizes the evaluations of the 15 aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances in this group.

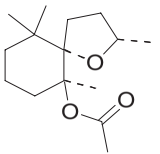
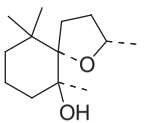
Table 4

Summary of the results of safety evaluations of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances 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					
2,3,4-Trimethyl-3-pentanol	1643	3054-92-0 	No Europe: 0.04 USA: ND Japan: ND	See note 1	No safety concern
(±)-2,4,8-Trimethyl-7-nonen-2-ol	1644	437770-28-0 	No Europe: 0.01 USA: 0.1 Japan: ND	See note 2	No safety concern
trans- and *cis*-2,4,8-Trimethyl-3,7-nonadien-2-ol	1645	479547-57-4 	No Europe: 0.01 USA: 0.1 Japan: ND	See note 2	No safety concern

Nerolidol	1646	7212-44-4 	No Europe: 38 USA: 23 Japan: 87	See note 2	No safety concern
1-Phenyl-3-methyl-3-pentanol	1649	10415-87-9 	No Europe: 0.01 USA: 0.1 Japan: 0.9	See note 1	No safety concern
p-α,α-Trimethylbenzyl alcohol	1650	1197-01-9 	No Europe: 9 USA: 0.01 Japan: 0.7	See note 1	No safety concern
(±)-Ethyl 2-hydroxy-2-methylbutyrate	1651	77-70-3 	No Europe: ND USA: 0.6 Japan: ND	See note 1	No safety concern
(±)-Ethyl 2-hydroxy-3-methylvalerate	1652	24323-38-4 	No Europe: ND USA: 0.2 Japan: ND	See note 1	No safety concern

α,α-Dimethylphenethyl alcohol	1653	100-86-7 	No Europe: 17 USA: 12 Japan: 16	See note 1	No safety concern
α,α-Dimethylphenethyl formate	1654	10058-43-2 	No Europe: ND USA: 0.4 Japan: 0.2	See note 3	No safety concern
α,α-Dimethylphenethyl acetate	1655	151-05-3 	No Europe: 41 USA: 574 Japan: 50	See note 3	No safety concern
α,α-Dimethylphenethyl butyrate	1656	10094-34-5 	No Europe: 93 USA: 1020 Japan: 98	See note 3	No safety concern
α,α-Dimethylbenzyl isobutyrate	1657	7774-60-9 	No Europe: 0.01 USA: ND Japan: 0.03	See note 3	No safety concern

Structural class II					
6-Acetoxydihydrotheaspirane	1647	57893-27-3 	No Europe: 0.07 USA: ND Japan: 0.03	See note 3	No safety concern
6-Hydroxydihydrotheaspirane	1648	65620-50-0 	No Europe: 0.1 USA: 0.05 Japan: 0.03	See note 3	No safety concern

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

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

[b] *Step 1*: Thirteen flavouring agents are in structural class I, and two (Nos 1647 and 1648) are in structural class II.

[c] *Step 2*: All of 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.

Notes to Table 4:

1. Tertiary alcohols are metabolized primarily by conjugation with glucuronic acid and excreted in the urine.
2. Tertiary unsaturated alcohols are metabolized primarily by conjugation with glucuronic acid and excreted in the urine. Oxidation of the allylic methyl group may occur at high doses.
3. Esters are hydrolysed, and the corresponding tertiary alcohols are metabolized primarily by conjugation with glucuronic acid and excreted in the urine.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are predicted to be metabolized by hydrolysis and/or oxidative metabolism of alcohol and aldehyde groups and alkyl side-chains, by conjugation reactions such as glucuronidation and possibly by aromatic hydroxylation. These pathways have a high capacity and would not be saturated, even if all agents were consumed at the same time. Some of the substances in this group that have been evaluated at this meeting and at the fifty-first and sixty-first meetings are predicted to be metabolized to common metabolites. Common metabolites (and their precursors) are linalool (Nos 356 and 358–365), terpineol (Nos 366–372), α,α-dimethylphenyl alcohol (Nos 1653–1657) and 6-hydroxydihydrotheaspirane (Nos 1647 and 1648). The estimated combined intake of substances predicted to be metabolized to α,α-dimethylphenyl alcohol was below the threshold for class I, and the combined intake of acetoxydihydrotheaspirane and 6-hydroxydihydrotheaspirane (Nos 1647 and 1648) was below the threshold for class II. In the case of linalool, the total combined intake did not add significantly to the combined intake of linalool (No. 356) and linalyl acetate (No. 359), which exceeded the class I threshold. This combined intake was evaluated at the fifty-first meeting and was considered not to be a safety concern. In the case of terpineol, the total combined intake did not add significantly to the combined intake of terpineol (No. 366) and terpinyl acetate (No. 368), which exceeded the class I threshold. This combined intake was evaluated at the fifty-first meeting and was considered not to be a safety concern. The Committee concluded that under the conditions of use as flavouring agents, the combined intake 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

Two members of this group of flavouring agents, *p*-α,α-trimethylbenzyl alcohol (No. 1650) and α,α-dimethylphenethyl formate (No. 1654), have assay values of <95%. Information on the safety of the secondary components of these two 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 of *p*-α,α-trimethylbenzyl alcohol, *p*-α-dimethylstyrene (No. 1333), is expected to undergo cytochrome P450-mediated metabolism followed by hydrolysis/conjugation. It is considered not to present a safety concern at current levels of intake. The secondary component of α,α-dimethylphenethyl formate, α,α-dimethylphenethyl alcohol, is expected to share the same metabolic fate as the ester and is considered not to present a safety concern at current levels of intake.

Conclusion

In the previous evaluations of substances in this group, studies of acute toxicity, short-term toxicity (84–149 days), carcinogenicity and genotoxicity were available. None raised safety concerns. The data available for this evaluation were supported by those from the previous evaluation.

The Committee concluded that these 15 flavouring agents, which are additions to the group of aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances evaluated previously, would not give rise to safety concerns at the currently estimated levels of intake.

An addendum to the toxicological monograph was prepared.

4.1.3 *Simple aliphatic and aromatic sulfides and thiols: additional compounds*

The Committee evaluated a group of flavouring substances consisting of 51 simple aliphatic and aromatic sulfides and thiols, which included 3 simple sulfides (Nos 1683, 1684 and 1707), 10 acyclic sulfides with oxidized and thiol side-chains (Nos 1668, 1675, 1677, 1688–1692, 1703 and 1710), 1 heterocyclic sulfide (No. 1685), 5 thiols (Nos 1659 and 1662–1665), 12 thiols with oxidized side-chains (Nos 1666, 1667, 1669–1674, 1704–1706 and 1708), 3 dithiols (Nos 1660, 1661 and 1709), 7 disulfides (Nos 1693, 1694 and 1696–1700), 2 trisulfides (Nos 1695 and 1701), 2 heterocyclic disulfides (Nos 1686 and 1687) and 6 thioesters and acids (Nos 1676, 1678–1681 and 1702). The evaluations were conducted according to the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these substances has previously been evaluated.

The Committee previously evaluated 137 other members of this chemical group of flavouring agents at its fifty-third meeting (Annex 1, reference *143*). The findings from these evaluations were considered in the present evaluation. All 137 substances in that group were concluded to be of no safety concern based on currently estimated levels of intake.

The Committee also evaluated 12 additional members of this chemical group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*). The findings from these evaluations were considered in the present evaluation. All 12 additional substances in that group were concluded to be of no safety concern based on currently estimated levels of intake.

Thirty-seven of the 51 flavouring agents in this group are natural components of foods (Nos 1659, 1660, 1662–1665, 1667, 1668, 1674, 1676–1678, 1680, 1683–1687, 1690–1704, 1706, 1707, 1709 and 1710). They have been detected primarily in beef, chicken, pork, fish, lobster, cheese, eggs, grapefruit

juice, coffee, cabbage, onion, scallions, garlic, potato, tomato, melon, papaya, pineapple, kiwifruit, chive, nobiru, kohlrabi, wakegi, leek, caucas, strawberry, hop oil, beer, wine, rum, filbert, hazelnut, arrack, fish oil, carrot, sauerkraut, trassi, sweet corn, sesame seed, passion fruit and durian fruit. Quantitative intake data were available for two substances, 1-pentanethiol (No. 1662) and methyl (methylthio)acetate (No. 1691). The consumption ratios (the ratios of their consumption from natural food sources to their use as flavouring agents) were calculated to be 106 and 37, respectively.

Assessment of dietary exposure

The total annual volume of production of the 51 simple aliphatic and aromatic sulfides and thiols is approximately 182 kg in Europe, 80 kg in the USA and 3 kg in Japan. In Europe, approximately 72% of the total volume is accounted for solely by diethyl trisulfide (No. 1701), whereas in the USA, (*S*)-1-methoxy-3-heptanethiol (No. 1671), (±)-isobutyl 3-methylthiobutyrate (No. 1677), methyl (methylthio)acetate (No. 1691), bis-(1-mercaptopropyl) sulfide (No. 1709) and *S*-allyl-L-cysteine (No. 1710) account for 84% of the total volume of production.

Absorption, distribution, metabolism and elimination

All of the sulfur-containing flavouring agents reviewed here are of low molecular weight and are sufficiently lipophilic to be absorbed. These flavouring agents are expected to be metabolized through the various pathways described below and in the previous evaluations by the Committee (Annex 1, references *143* and *166*).

a) Simple sulfides (Nos 1683, 1684 and 1707). Once simple sulfides (thioethers) enter the systemic circulation, they are rapidly oxidized to sulfoxides and, depending on the structure of the thioether, may be further oxidized to sulfones. Aliphatic thioethers yield mixtures of sulfoxide and sulfone urinary metabolites. Enzymes of the cytochrome P450 superfamily and flavin-containing monooxygenases catalyse the oxidation of thioethers to sulfoxides. Oxidation of sulfoxides to the corresponding sulfones occurs both in tissues and in aerobic microorganisms and is an irreversible metabolic reaction in mammals. Sulfoxides can also be metabolized back to the thioether by thioredoxin and its reductase and by the gut microflora in the anaerobic environment of the lower bowel.

b) Acyclic sulfides with oxidized or thiol side-chains (Nos 1668, 1675, 1677, 1688–1692, 1703 and 1710). Hemiacetal derivatives of thiols (No. 1675) would be expected to undergo metabolism via the pathways described above for simple thiols. The thioether sulfur and the free thiol can undergo oxidation as described in more detail below, and methylation of the free thiol could also

occur. The presence of oxygenated functional groups, such as an alcohol (No. 1703), aldehyde (No. 1692), acid (No. 1710), β-ketone (Nos 1688 and 1689) or ester (Nos 1668, 1677, 1690 and 1691), provides additional sites for biotransformation of sulfides, and the presence of these polar sites would result in increased renal excretion of these substances. The biotransformation of such oxygenated groups is well characterized and has been described for groups of flavouring agents evaluated previously by the Committee (Annex 1, references *131*, *132*, *138* and *144*). Simultaneous metabolism of sulfur and oxygenated functional groups has been reported for various substrates. Sulfoxide formation is usually the predominant metabolic detoxication pathway for sulfides.

c) Heterocyclic sulfide (No. 1685). Methyl-substituted cyclic sulfides can be expected to undergo oxidation by cytochrome P450 enzymes to produce the corresponding sulfoxides. The mono-sulfoxides are predicted to be the main urinary metabolites of simple cyclic sulfides.

d) Thiols (Nos 1659 and 1662–1665). Thiols are highly reactive in vivo, which is mainly due to the fact that most thiols exist in the ionized form at physiological pH. The biotransformation pathways of thiols include oxidation to unstable sulfenic acid (RSOH), which may be oxidized to the corresponding sulfinic acid (RSO_2H) and sulfonic acid (RSO_3H); methylation to yield methyl sulfides, which can be oxidized to methyl sulfoxides and sulfones; reaction with endogenous thiols such as glutathione and cysteine to form mixed disulfides; conjugation with glucuronic acid; and oxidation of the α-carbon, which results in desulfuration and the formation of an aldehyde intermediate. There are several possible thiol–disulfide exchange reactions that may occur, and they all result from nucleophilic substitution.

e) Thiols with oxidized side-chains (Nos 1666, 1667, 1669–1674, 1704–1706 and 1708). The metabolism of thiols with oxidized side-chains is predicted to involve a combination of pathways described above for simple thiols, together with further oxidation or conjugation of the oxidized side-chain.

f) Dithiols (Nos 1660, 1661 and 1709). Although they are more stable than hydrates, simple geminal dithiols (Nos 1660 and 1661) can undergo hydrolysis to yield their parent aldehydes and to release hydrogen sulfide. The metabolism of the other simple aliphatic dithiol (No. 1709) is predicted to involve the pathways described above for simple thiols. Urinary metabolites could result from methylation, *S*-oxidation of a sulfur atom to yield a polar sulfonate and the formation of mixed disulfides by combination with a low molecular weight endogenous thiol such as cysteine.

g) Simple disulfides (Nos 1693, 1694 and 1696–1700). The reduction of xenobiotic disulfides is believed to be extensive and can be catalysed

enzymatically by glutathione reductase or thioltransferases, as well as chemically by exchange with glutathione, thioredoxin, cysteine or other endogenous thiols. Reduction of non-cyclic disulfides results in the formation of thiols of low molecular weight that are metabolized via the various pathways described above for simple thiols.

h) Trisulfides (Nos 1695 and 1701). Trisulfides are predicted to be converted rapidly to the corresponding disulfides with subsequent reduction to thiols, which are then metabolized via the various pathways described above for simple thiols.

i) Heterocyclic disulfides (Nos 1686 and 1687). Heterocyclic disulfides are five- and six-carbon rings, which may also contain a cyclic thioether bond. The principal metabolic pathways are predicted to be disulfide reduction with ring opening to produce a dithiol and *S*-oxidation of the cyclic thioether.

j) Thioesters and acids (Nos 1676, 1678–1681 and 1702). Thioesters are hydrolysed by lipases and esterases; the rate of hydrolysis increases as the length of the carbon chain increases and decreases as the oxygenation of the carbon chain in the thiol moiety increases. After hydrolysis, the resulting alcohol and carboxylic acid would participate in the metabolic pathways described above for sulfides containing oxygenated functional groups.

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 these 51 flavouring agents, the Committee assigned 40 (Nos 1659–1664, 1667–1671, 1674–1679, 1683–1685, 1688–1699, 1701 and 1703–1709) to structural class I and 6 (Nos 1665, 1673, 1681, 1686, 1687 and 1700) to structural class II. The remaining 5 flavouring agents (Nos 1666, 1672, 1680, 1702 and 1710) were assigned to structural class III.

Step 2. None of the flavouring agents in this group can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the B-side of the Procedure.

Step B3. The estimated daily per capita intakes of the 40 flavouring agents in this group 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 6 flavouring agents in structural class II are below the threshold of concern (i.e. 540 μg/person per day for class II). The estimated daily per capita intakes of the 5 flavouring agents in structural class III are below the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of all 51 substances in the group proceeded to step B4.

Step B4. For 2-methyl-1-methylthio-2-butene (No. 1683), the NOEL of 250 mg/kg bw per day for the structurally related substance methyl sulfide (No. 452) from a 98-day study in male and female rats provides an adequate margin of safety (at least 125 million) in relation to currently estimated levels of intake of this substance from its use as a flavouring substance. This NOEL is also appropriate for the structurally related substances 2,4,6-trithiaheptane (No. 1684) and 2,5-dithiahexane (No. 1707), because they are all simple sulfides that are anticipated to undergo oxidation and subsequent metabolism via similar metabolic pathways. In relation to the currently estimated levels of intake from use as flavouring substances, the NOEL of 250 mg/kg bw provides adequate margins of safety of >1 billion[8] and 125 million for 2,4,6-trithiaheptane (No. 1684) and 2,5-dithiahexane (No. 1707), respectively.

For methionyl butyrate (No. 1668), the NOEL of 1.4 mg/kg bw per day for the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) from a 92-day study in male rats provides an adequate margin of safety (7 million) in relation to currently estimated levels of intake of this substance from its use as a flavouring substance. This NOEL is also appropriate for the structurally related substances (±)-isobutyl 3-methylthiobutyrate (No. 1677), methyl (methylthio)acetate (No. 1691) and (±)-3-(methylthio) heptanal (No. 1692), because they are all acyclic sulfides with oxidized side-chains. For these structurally related substances, the NOEL of 1.4 mg/kg bw provides adequate margins of safety in the range of 28 000 to 7 million in relation to the currently estimated levels of intake from use as flavouring agents.

For methylthiomethylmercaptan (No. 1675), the NOEL of 0.3 mg/kg bw per day for the structurally related substance 3-methyl-1,2,4-trithiane (No. 574) from a 90-day study in rats provides an adequate margin of safety (at least 150 000) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For 3-(methylthio)-2-butanone (No. 1688) and (±)-3-(ethylthio)butanol (No. 1703), the NOEL of 0.7 mg/kg bw per day for the structurally related substance 2-mercapto-3-butanol (No. 546) from a 90-day study in rats provides adequate margins of safety (>3 million and 350 000, respectively) in relation to estimated levels of intake of these substances from their use as flavouring agents.

For 4-(methylthio)-2-pentanone (No. 1689), the NOEL of 1.9 mg/kg bw per day for the structurally related substance 3-mercapto-2-pentanone (No. 560) from a 90-day study in rats provides an adequate margin of safety (>9 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

[8] Note that billion is defined as a thousand million (10^9).

For methyl 3-(methylthio)butanoate (No. 1690), the NOEL of 6.5 mg/kg bw per day for the structurally related substance ethyl thioacetate (No. 483) from a 91-day study in rats provides an adequate margin of safety (>32 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For *S*-allyl-L-cysteine (No. 1710), the NOEL of 250 mg/kg bw per day from a 28-day study in rats provides an adequate margin of safety (>8 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For (±)-2,8-epithio-*cis*-*p*-menthane (No. 1685), the NOEL of 10 mg/kg bw per day in female rats from a 28-day study provides an adequate margin of safety (>1 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For ethanethiol (No. 1659), the NOEL of 0.56 mg/kg bw per day for the structurally related substance cyclopentanethiol (No. 516) from a 90-day study in male and female rats provides an adequate margin of safety (at least 80 000) in relation to currently estimated levels of intake of this substance from use as a flavouring agent. This NOEL is also appropriate for the structurally related substances 1-pentanethiol (No. 1662), 1-heptanethiol (No. 1663) and 2-heptanethiol (No. 1664), because they are all simple thiols. For these structurally related substances, the NOEL of 0.56 mg/kg bw provides adequate margins of safety in the range of >100 000 to >2 million in relation to the currently estimated levels of intake from use as flavouring agents.

For (±)-1-phenylethylmercaptan (No. 1665), the NOEL of 0.43 mg/kg bw per day for the structurally related substance 2,6-dimethylthiophenol (No. 530) from a 90-day study in rats provides an adequate margin of safety (>2 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For propyl 2-mercaptopropionate (No. 1667), the NOEL of 0.7 mg/kg bw per day for the structurally related substance 2-mercapto-3-butanol (No. 546) from a 90-day study in rats provides an adequate margin of safety (at least 350 000) in relation to currently estimated levels of intake of this substance from use as a flavouring agent. This NOEL is also appropriate for the structurally related substances (±)-4-mercapto-4-methyl-2-pentanol (No. 1669), (*S*)-1-methoxy-3-heptanethiol (No. 1671), methyl 3-mercaptobutanoate (No. 1674), hexyl 3-mercaptobutanoate (No. 1704), (±)-3-mercapto-1-butyl acetate (No. 1705), 3-mercapto-3-methyl-1-butyl acetate (No. 1706), 3-mercaptoheptyl acetate (No. 1708) and *cis*- and *trans*-l-mercapto-*p*-menthan-3-one (No. 1673), because they are all thiols with oxidized side-chains. For these structurally related substances, the NOEL of 0.7 mg/kg bw provides

adequate margins of safety in the range of >23 000 to >3 million in relation to the currently estimated intakes from use as flavouring agents.

For 4-mercapto-2-pentanone (No. 1670), the NOEL of 1.9 mg/kg bw per day for the structurally related substance 3-mercapto-2-pentanone (No. 560) from a 90-day study in rats provides an adequate margin of safety (>1 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For 2-mercaptoanisole (No. 1666), the NOEL of 0.51 mg/kg bw per day for the structurally related substance 2-mercaptomethylbenzene (No. 528) from a 90-day study in rats provides an adequate margin of safety (at least 25 500) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For ethane-1,1-dithiol (No. 1660), the NOEL of 125 mg/kg bw per day for one hydrolysis product, acetaldehyde (No. 80), from a 28-day study in rats and the NOEL of 6.5 mg/kg bw per day for the other hydrolysis product, hydrogen sulfide, from a 90-day inhalation study in rats provide adequate margins of safety (625 million and >32 million, respectively) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For dimercaptomethane (No. 1661), the NOEL of 15 mg/kg bw per day for one hydrolysis product, formaldehyde, from a 2-year study in rats and the NOEL of 6.5 mg/kg bw per day for the other hydrolysis product, hydrogen sulfide, from a 90-day inhalation study in rats provide adequate margins of safety (75 million and >32 million, respectively) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For bis(1-mercaptopropyl)sulfide (No. 1709), the NOEL of 0.7 mg/kg bw per day for the structurally related substance 2,3-butanedithiol (No. 539) from a 90-day study in rats provides an adequate margin of safety (70 000) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For ethyl methyl disulfide (No. 1693), the NOEL of 7.3 mg/kg bw per day for the structurally related substance propyl disulfide (No. 566) from a 90-day study in rats provides an adequate margin of safety (>14 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent. This NOEL is also appropriate for the structurally related substances ethyl propyl disulfide (No. 1694), methyl isopentyl disulfide (No. 1696), amyl methyl disulfide (No. 1697), butyl ethyl disulfide (No. 1698) and diethyl disulfide (No. 1699), because they are all simple disulfides. For these structurally related substances, the NOEL of 7.3 mg/kg bw provides adequate margins of safety in the range of >14 million to >36 million in

relation to the currently estimated intakes of these substances from use as flavouring agents.

For allyl propyl disulfide (No. 1700), the NOEL of 4.6 mg/kg bw per day for the structurally related substance diallyl trisulfide (No. 587) from a 90-day study in rats provides an adequate margin of safety (>4 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For ethyl propyl trisulfide (No. 1695), the NOEL of 4.8 mg/kg bw per day for the structurally related substance dipropyl trisulfide (No. 585) from a 90-day study in rats provides an adequate margin of safety (24 million) in relation to currently estimated levels of intake of this substance from use as a flavouring agent. This NOEL is also appropriate for the structurally related substance diethyl trisulfide (No. 1701), because it is also a trisulfide. The NOEL of 4.8 mg/kg bw per day provides an adequate margin of safety of 24 000 for this substance in relation to the currently estimated level of intake from use as a flavouring agent.

For 3,5-diethyl-1,2,4-trithiolane (No. 1686), the NOEL of 1.9 mg/kg bw per day for the structurally related substance 3,5-dimethyl-1,2,4-trithiolane (No. 573) from a 91-day study in rats provides an adequate margin of safety (at least 190 000) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For the mixture of 3,6-diethyl-1,2,4,5-tetrathiane (approximately 55%) and 3,5-diethyl-1,2,4-trithiolane (approximately 45%) (No. 1687), the NOEL of 0.3 mg/kg bw per day for the structurally related substance 3-methyl-1,2,4-trithiane (No. 574) from a 90-day study in rats provides an adequate margin of safety (30 000) in relation to currently estimated levels of intake of this substance from use as a flavouring agent.

For thioacetic acid (No. 1676), the NOEL of 6.5 mg/kg bw per day for the structurally related substance ethyl thioacetate (No. 483) from a 91-day study in rats provides an adequate margin of safety (>900 000) in relation to currently estimated levels of intake of this substance from use as a flavouring agent. This NOEL is also appropriate for the structurally related substances *S*-methyl propanethioate (No. 1678), *S*-isopropyl 3-methylbut-2-enethioate (No. 1679), allyl thiohexanoate (No. 1681) and *S*-ethyl 2-acetylaminoethanethioate (No. 1680), because they are all thioesters and related acids. For these structurally related substances, the NOEL of 6.5 mg/kg bw per day provides adequate margins of safety in the range of >3 million to >32 million in relation to their currently estimated levels of intake from their use as flavouring agents.

Step B5. Two substances, diisopentyl thiomalate (No. 1672) and propyl propane thiosulfonate (No. 1702), were evaluated at this step of the Procedure.

The currently estimated daily per capita intakes of both substances are below 1.5 µg/person per day in Europe. Applying the criteria for step B5 outlined in Annex 5 of the evaluations published after its forty-ninth meeting (Annex 1, reference *131*), the Committee concluded that the use of these substances as flavouring agents at their currently estimated levels of intake poses no safety concern.

Table 5 summarizes the evaluations of the 51 simple aliphatic and aromatic sulfides and thiols in this group.

Consideration of combined intakes from use as flavouring agents

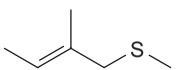
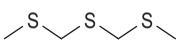
The substances in this group that have been evaluated at this meeting and at the fifty-third and sixty-first meetings are predicted to be metabolized by a variety of metabolic pathways. Because of the diverse structures, there are few common metabolites. Examples are 3-(methylthio)propionic acid (from Nos 476 and 468) and thioacetic acid (from Nos 482, 483, 485 and 491). The combined intakes of substances with a common metabolite were below the relevant TTC value.

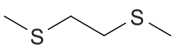
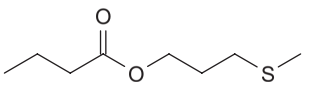
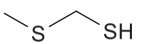
Several substances in this group contain a sulfide group, which would be metabolized by *S*-oxidation. These include simple sulfides and sulfoxides for which *S*-oxidation would be the main route of metabolism (Nos 452–460, 507, 533, 1683, 1684, 1685 and 1707), acyclic sulfides with oxidized side-chains, which would have alternative processes of elimination (Nos 461–463, 465–481, 495–503, 505, 1297, 1298, 1668, 1677, 1688–1692, 1703 and 1710) and cyclic sulfides, some of which have additional functional groups, providing alternative processes of elimination (Nos 456, 464, 498, 499, 534, 543, 550, 563, 1296 and 1685). The majority of the combined intake of all compounds was from methyl sulfide (No. 452). The Committee concluded that under the current conditions of use as flavouring agents, the combined intake of these substances would not saturate *S*-oxidation and combined intakes would not raise safety concerns.

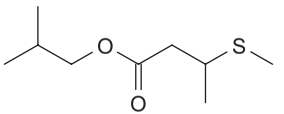
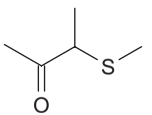
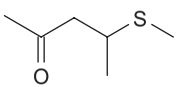
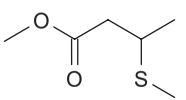
A number of substances in this group contain a thiol group, which is predicted to be metabolized by methylation followed by oxidation, conjugation with glutathione, *S*-glucuronidation and/or oxidation to sulfonic acids. These would be the major routes of elimination of simple alkyl and aryl thiols (Nos 508–531, 1659 and 1662–1665) and dithiols (Nos 532, 535–542, 1660, 1661 and 1709), whereas alternative processes of elimination would be available for thiols with additional functional groups (Nos 544–549, 551–561, 563, 1289–1294, 1666, 1667, 1669–1675, 1704–1706 and 1708). The Committee concluded that under the current conditions of their use as flavouring agents, the combined intake of these substances would not saturate the metabolic pathways and combined intakes would not raise safety concerns.

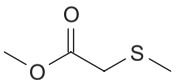
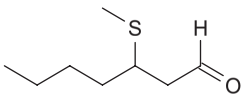
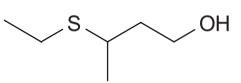
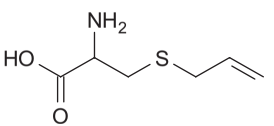
Table 5

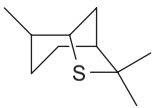
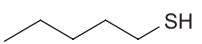
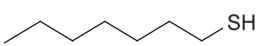
Summary of the results of safety evaluations of simple aliphatic and aromatic sulfides and thiols used as flavouring agents[a,b,c]

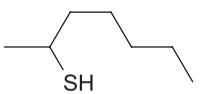
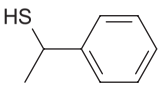
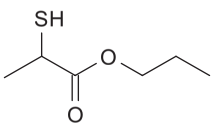
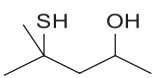
Flavouring agent	No.	CAS No. and structure	*Step B3*[d] Does intake exceed the threshold for human intake?	*Step B4* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Simple sulfides *Structural class I*						
2-Methyl-1-methylthio-2-butene	1683	89534-74-7 	No Europe: 0.01 USA: 0.1 Japan: ND	Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) is at least 125 million times the estimated daily intake of 2-methyl-1-methylthio-2-butene when used as a flavouring agent.	See note 1	No safety concern
2,4,6-Trithiaheptane	1684	6540-86-9 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) is >1 billion times the estimated daily intake of 2,4,6-trithiaheptane when used as a flavouring agent.	See note 1	No safety concern

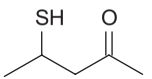
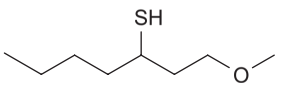
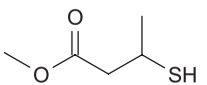
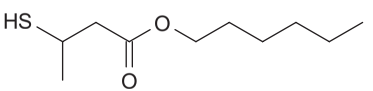
2,5-Dithiahexane	1707	6628-18-8 	No Europe: ND USA: 0.1 Japan: ND	Yes. The NOEL of 250 mg/kg bw per day for the related substance methyl sulfide (No. 452) is 125 million times the estimated daily intake of 2,5-dithiahexane when used as a flavouring agent.	See note 1	No safety concern
Acyclic sulfides with oxidized and thiol side-chains *Structural class I*						
Methionyl butyrate	1668	16630-60-7 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is 7 million times the estimated daily intake of methionyl butyrate when used as a flavouring agent.	See notes 1, 2 and 3	No safety concern
Methylthiomethylmercaptan	1675	29414-47-9 	No Europe: 0.01 USA: 0.1 Japan: ND	Yes. The NOEL of 0.3 mg/kg bw per day for the related substance 3-methyl-1,2,4-trithiane (No. 574) is at least 150 000 times the estimated daily intake of methylthiomethylmercaptan when used as a flavouring agent.	See note 8	No safety concern

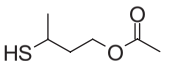
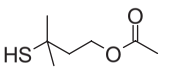
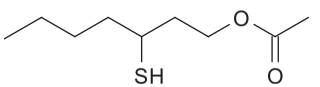
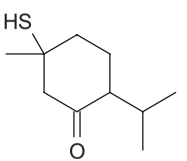
(±)-Isobutyl 3-methylthiobutyrate	1677	127931-21-9		No Europe: 0.2 USA: 3 Japan: ND	Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 28 000 times the estimated daily intake of (±)-isobutyl 3-methylthiobutyrate when used as a flavouring agent.	See notes 1 and 2	No safety concern
3-(Methylthio)-2-butanone	1688	53475-15-3		No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is >3 million times the estimated daily intake of 3-(methylthio)-2-butanone when used as a flavouring agent.	See notes 1 and 4	No safety concern
4-(Methylthio)-2-pentanone	1689	143764-28-7		No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 1.9 mg/kg bw per day for the related substance 3-mercapto-2-pentanone (No. 560) is >9 million times the estimated daily intake of 4-(methylthio)-2-pentanone when used as a flavouring agent.	See notes 1 and 4	No safety concern
Methyl 3-(methylthio)butanoate	1690	207983-28-6		No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) is >32 million times the estimated daily intake of methyl 3-(methylthio) butanoate when used as a flavouring agent.	See notes 1 and 2	No safety concern

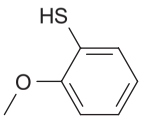
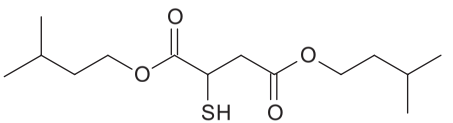
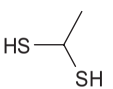
Methyl (methylthio)acetate	1691	16630-66-3 	No Europe: 0.1 USA: 1 Japan: 0.1	Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is at least 70 000 times the estimated daily intake of methyl (methylthio) acetate when used as a flavouring agent.	See notes 1 and 2	No safety concern
(±)-3-(Methylthio)heptanal	1692	51755-70-5 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 1.4 mg/kg bw per day for the related substance 2-(methylthiomethyl)-3-phenylpropenal (No. 505) is 7 million times the estimated daily intake of (±)-3-(methylthio) heptanal when used as a flavouring agent.	See notes 1 and 5	No safety concern
(±)-3-(Ethylthio)butanol	1703	117013-33-9 	No Europe: ND USA: 0.1 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is 350 000 times the estimated daily intake of (±)-3-(ethylthio)butanol when used as a flavouring agent.	See notes 1 and 3	No safety concern
Structural class III						
S-Allyl-L-cysteine	1710	21593-77-1 	No Europe: ND USA: 2 Japan: ND	Yes. The NOEL of 250 mg/kg bw per day is >8 million times the estimated daily intake of *S*-allyl-L-cysteine when used as a flavouring agent.	See notes 1 and 2	No safety concern

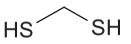
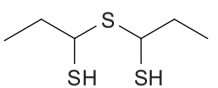
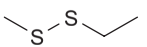
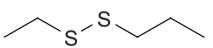
Heterocyclic sulfides *Structural class I*						
(±)-2,8-Epithio-*cis*-*p*-menthane	1685	68398-18-5 	No Europe: 0.4 USA: ND Japan: 0.03	Yes. The NOEL of 10 mg/kg bw per day is >1 million times the estimated daily intake of (±)-2,8-epithio-*cis*-*p*-menthane when used as a flavouring agent.	See note 6	No safety concern
Simple thiols *Structural class I*						
Ethanethiol	1659	75-08-1 	No Europe: 0.4 USA: ND Japan: 0.05	Yes. The NOEL of 0.56 mg/kg bw per day for the related substance cyclopentanethiol (No. 516) is at least 80 000 times the estimated daily intake of ethanethiol when used as a flavouring agent.	See note 8	No safety concern
1-Pentanethiol	1662	110-66-7 	No Europe: 0.05 USA: 0.2 Japan: 0.05	Yes. The NOEL of 0.56 mg/kg bw per day for the related substance cyclopentanethiol (No. 516) is >100 000 times the estimated daily intake of 1-pentanethiol when used as a flavouring agent.	See note 8	No safety concern
1-Heptanethiol	1663	1639-09-4 	No Europe: 0.03 USA: ND Japan: ND	Yes. The NOEL of 0.56 mg/kg bw per day for the related substance cyclopentanethiol (No. 516) is >1 million times the estimated daily intake of 1-heptanethiol when used as a flavouring agent.	See note 8	No safety concern

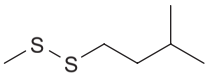
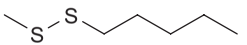
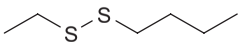
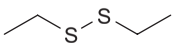
2-Heptanethiol	1664	628-00-2 	No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 0.56 mg/kg bw per day for the related substance cyclopentanethiol (No. 516) is >2 million times the estimated daily intake of 2-heptanethiol when used as a flavouring agent.	See note 8	No safety concern
Structural class II						
(±)-1-Phenylethylmercaptan	1665	6263-65-6 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 0.43 mg/kg bw per day for the related substance 2,6-dimethylthiophenol (No. 530) is >2 million times the estimated daily intake of (±)-1-phenylethylmercaptan when used as a flavouring agent.	See note 8	No safety concern
Thiols with oxidized side-chains						
Structural class I						
Propyl 2-mercaptopropionate	1667	19788-50-2 	No Europe: 0.01 USA: 0.1 Japan: 0.03	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is at least 350 000 times the estimated daily intake of propyl 2-mercaptopropionate when used as a flavouring agent.	See notes 2 and 8	No safety concern
(±)-4-Mercapto-4-methyl-2-pentanol	1669	31539-84-1 	No Europe: 0.01 USA: 0.1 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is at least 350 000 times the estimated daily intake of (±)-4-mercapto-4-methyl-2-pentanol when used as a flavouring agent.	See notes 3 and 8	No safety concern

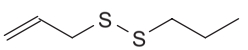
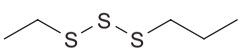
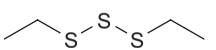
4-Mercapto-2-pentanone	1670	92585-08-5 	No Europe: ND USA: 0.07 Japan: ND	Yes. The NOEL of 1.9 mg/kg bw per day for the related substance 3-mercapto-2-pentanone (No. 560) is >1 million times the estimated daily intake of 4-mercapto-2-pentanone when used as a flavouring agent.	See notes 4 and 8	No safety concern
(*S*)-1-Methoxy-3-heptanethiol	1671	400052-49-5 	No Europe: 0.01 USA: 2 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is >23 000 times the estimated daily intake of (*S*)-1-methoxy-3-heptanethiol when used as a flavouring agent.	See notes 2, 3 and 8	No safety concern
Methyl 3-mercaptobutanoate	1674	54051-19-3 	No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is >3 million times the estimated daily intake of methyl 3-mercaptobutanoate when used as a flavouring agent.	See notes 2, 3 and 8	No safety concern
Hexyl 3-mercaptobutanoate	1704	796857-79-9 	No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is >3 million times the estimated daily intake of hexyl 3-mercaptobutanoate when used as a flavouring agent.	See notes 2 and 8	No safety concern

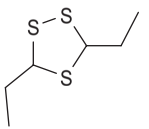
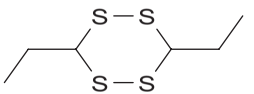
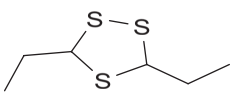
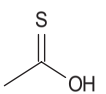
(±)-3-Mercapto-1-butyl acetate	1705	89534-38-3 	No Europe: ND USA: 0.1 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is 350 000 times the estimated daily intake of (±)-3-mercapto-1-butyl acetate when used as a flavouring agent.	See notes 2, 3 and 8	No safety concern
3-Mercapto-3-methyl-1-butyl acetate	1706	50746-09-3 	No Europe: ND USA: 0.1 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is 350 000 times the estimated daily intake of 3-mercapto-3-methyl-1-butyl acetate when used as a flavouring agent.	See notes 2, 3 and 8	No safety concern
3-Mercaptoheptyl acetate	1708	548774-80-7 	No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is >3 million times the estimated daily intake of 3-mercaptoheptyl acetate when used as a flavouring agent.	See notes 2, 3 and 8	No safety concern
Structural class II						
cis- and *trans*-Mercapto-*p*-menthan-3-one	1673	29725-66-4 	No Europe: 1 USA: ND Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2-mercapto-3-butanol (No. 546) is 35 000 times the estimated daily intake of *cis*- and *trans*-mercapto-*p*-menthan-3-one when used as a flavouring agent.	See notes 4 and 8	No safety concern

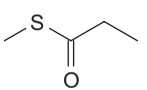
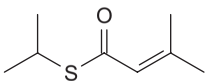
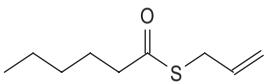
Structural class III							
2-Mercaptoanisole	1666	7217-59-6		No Europe: 1 USA: ND Japan: 0.03	The NOEL of 0.51 mg/kg bw per day for the related substance 2-mercaptomethylbenzene (No. 528) is at least 25 500 times the estimated daily intake of 2-mercaptoanisole when used as a flavouring agent.	See notes 2, 3 and 8	No safety concern
Diisopentyl thiomalate	1672	68084-03-7		No Europe: 0.01 USA: ND Japan: ND	No, proceeded to step B5	See notes 2, 3 and 8	No safety concern[e]
Dithiols							
Structural class I							
Ethane-1,1-dithiol	1660	69382-62-3		No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOELs of 125 mg/kg bw per day and 6.5 mg/kg bw per day for the hydrolysis products acetaldehyde (No. 80) and hydrogen sulfide, respectively, are 625 million and >32 million times the estimated daily intake of ethane-1,1-dithiol when used as a flavouring agent.	See note 8	No safety concern

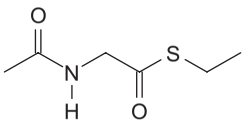
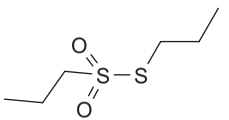
Dimercaptomethane	1661	6725-64-0 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOELs of 15 mg/kg bw per day and 6.5 mg/kg bw per day for the hydrolysis products formaldehyde and hydrogen sulfide, respectively, are 75 million and >32 million times the estimated daily intake of dimercaptomethane when used as a flavouring agent.	See note 8	No safety concern
bis(1-Mercaptopropyl)sulfide	1709	53897-60-2 	No Europe: ND USA: 0.6 Japan: ND	Yes. The NOEL of 0.7 mg/kg bw per day for the related substance 2,3-butanedithiol (No. 539) is 70 000 times the estimated daily intake of bis(1-mercaptopropyl) sulfide when used as a flavouring agent.	See notes 1 and 8	No safety concern
Simple disulfides *Structural class I*						
Ethyl methyl disulfide	1693	20333-39-5 	No Europe: 0.01 USA: ND Japan: 0.03	Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is >14 million times the estimated daily intake of ethyl methyl disulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern
Ethyl propyl disulfide	1694	30453-31-7 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is >36 million times the estimated daily intake of ethyl propyl disulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern

Methyl isopentyl disulfide	1696	72437-56-0		No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is >36 million times the estimated daily intake of methyl isopentyl disulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern
Amyl methyl disulfide	1697	72437-68-4		No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is >36 million times the estimated daily intake of amyl methyl disulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern
Butyl ethyl disulfide	1698	63986-03-8		No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is >36 million times the estimated daily intake of butyl ethyl disulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern
Diethyl disulfide	1699	110-81-6		No Europe: 0.01 USA: ND Japan: 0.03	Yes. The NOEL of 7.3 mg/kg bw per day for the related substance propyl disulfide (No. 566) is >14 million times the estimated daily intake of diethyl disulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern

Structural class II						
Allyl propyl disulfide	1700	2179-59-1 	No Europe: 0.03 USA: ND Japan: 0.08	Yes. The NOEL of 4.6 mg/kg bw per day for the related substance diallyl trisulfide (No. 587) is >4 million times the estimated daily intake of allyl propyl disulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern
Trisulfides *Structural class I*						
Ethyl propyl trisulfide	1695	31499-70-4 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 4.8 mg/kg bw per day for the related substance dipropyl trisulfide (No. 585) is 24 million times the estimated daily intake of ethyl propyl trisulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern
Diethyl trisulfide	1701	3600-24-6 	No Europe: 14 USA: ND Japan: ND	Yes. The NOEL of 4.8 mg/kg bw per day for the related substance dipropyl trisulfide (No. 585) is 24 000 times the estimated daily intake of diethyl trisulfide when used as a flavouring agent.	See notes 9 and 10	No safety concern

Heterocyclic disulfides *Structural class II*						
3,5-Diethyl-1,2,4-trithiolane	1686	54644-28-9 	No Europe: 0.6 USA: 0.01 Japan: 0.03	Yes. The NOEL of 1.9 mg/kg bw per day for the related substance 3,5-dimethyl-1,2,4-trithiolane (No. 573) is at least 190 000 times the estimated daily intake of 3,5-diethyl-1,2,4-trithiolane when used as a flavouring agent.	See note 11	No safety concern
Mixture of 3,6-diethyl-1,2,4,5-tetrathiane (approximately 55%) and 3,5-diethyl-1,2,4-trithiolane (approximately 45%)	1687	54717-12-3 54644-28-9  	No Europe: 0.6 USA: ND Japan: ND	Yes. The NOEL of 0.3 mg/kg bw per day for the related substance 3-methyl-1,2,4-trithiane (No. 574) is 30 000 times the estimated daily intake of the mixture of 3,6-diethyl-1,2,4,5-tetrathiane and 3,5-diethyl-1,2,4-trithiolane when used as a flavouring agent.	See note 11	No safety concern
Thioesters and acids *Structural class I*						
Thioacetic acid	1676	507-09-5 	No Europe: 0.2 USA: ND Japan: 0.4	Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) is >900 000 times the estimated daily intake of thioacetic acid when used as a flavouring agent.	See note 12	No safety concern

S-Methyl propanethioate	1678	5925-75-7 	No Europe: 0.01 USA: 0.1 Japan: 0.03	Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) is >3 million times the estimated daily intake of *S*-methyl propanethioate when used as a flavouring agent.	See note 12	No safety concern
S-Isopropyl 3-methylbut-2-enethioate	1679	34365-79-2 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) is >32 million times the estimated daily intake of *S*-isopropyl 3-methylbut-2-enethioate when used as a flavouring agent.	See note 12	No safety concern
Structural class II						
Allyl thiohexanoate	1681	156420-69-8 	No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) is >32 million times the estimated daily intake of allyl thiohexanoate when used as a flavouring agent.	See note 12	No safety concern

Structural class III							
S-Ethyl 2-acetylaminoethanethioate	1680	4396-62-7 		No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 6.5 mg/kg bw per day for the related substance ethyl thioacetate (No. 483) is >32 million times the estimated daily intake of *S*-ethyl 2-acetylaminoethanethioate when used as a flavouring agent.	See note 12	No safety concern
Propyl propane thiosulfonate	1702	1113-13-9 		No Europe: 0.01 USA: ND Japan: ND	No, proceeded to step 5	See note 12	No safety concern[e]

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

[a] One hundred and forty-nine flavouring agents in this group were previously evaluated by the Committee (Annex 1, references *143* and *166*). To facilitate the evaluations, the group was divided into 12 subgroups based on the position of the sulfur atom. The subgroup designations are indicated in the table.

[b] *Step 1*: Forty flavouring agents are in structural class I, six are in structural class II and five are in structural class III.

[c] S*tep 2*: None of the agents in this group can be predicted to be metabolized to innocuous products.

[d] The thresholds for human combined per capita intake for structural classes I, II and III are 1800, 540 and 90 µg/person per day, respectively. All intake values are expressed in µg/day.

[e] *Step B5*: Conditions of use do not result in an exposure greater than 1.5 µg/day; therefore, the substance is not expected to be a safety concern.

Notes to Table 5:

1. The sulfur is expected to be oxidized to the sulfoxide and sulfone.
2. The ester is expected to undergo hydrolysis to the corresponding carboxylic acid and alcohol.
3. The hydroxy group is expected to undergo oxidation to the carboxylic acid.
4. The ketone group is expected to be reduced to the alcohol, conjugated and subsequently excreted.
5. The aldehyde group is expected to be oxidized to the corresponding carboxylic acid, conjugated and subsequently excreted.
6. The sulfur is expected to be oxidized to the sulfoxide.
7. Sulfur is expected to undergo oxidative desulfuration to yield an aldehyde intermediate.

8. Sulfur is expected to be oxidized to sulfonic acid, undergo alkylation and conjugation and be excreted.
9. The di- or trisulfides are expected to be reduced to free thiols.
10. Free thiols may form mixed disulfides with glutathione or cysteine.
11. The heterocyclic disulfide is expected to undergo reduction to produce a dithiol and oxidation of the cyclic thioether.
12. The thioester is expected to undergo hydrolysis to acetate and the corresponding thiol, which will be further oxidized.

A number of substances contain a disulfide or polysulfide group, which is predicted to be biotransformed initially by reduction to thiols, which would then be metabolized as described above. Some substances were simple alkyl and aryl disulfides or polysulfides (Nos 564–579, 582–588, 1299, 1300, 1686, 1687 and 1693–1701), whereas alternative processes of elimination would be available for disulfides or polysulfides with additional functional groups (Nos 580 and 581). The Committee concluded that under the current conditions of use as flavouring agents, the combined intake of these substances would not saturate the metabolic pathways and combined intakes would not raise safety concerns.

Some substances were thioic acids (No. 1676) and their esters (Nos 482–494, 504, 506, 1295 and 1678–1681), which are predicted to be eliminated by conversion to the oxy-analogue and/or excretion as the thioic acid. Thioesters (Nos 482–494, 504, 506, 1295 and 1678–1681) would be hydrolysed to the corresponding thioic acid prior to elimination. The Committee concluded that under the current conditions of use as flavouring agents, the combined intake of these substances would not saturate the metabolic pathways and combined intakes would not raise safety concerns.

Consideration of secondary components

Fourteen members of this group of flavouring substances, ethane-1,1-dithiol (No. 1660), 4-mercapto-2-pentanone (No. 1670), diisopentyl thiomalate (No. 1672), *cis*- and *trans*-l-mercapto-*p*-menthan-3-one (No. 1673), 2,4,6-trithiaheptane (No. 1684), (±)-2,8-epithio-*cis*-*p*-menthane (No. 1685), mixture of 3,6-diethyl-1,2,4,5-tetrathiane and 3,5-diethyl-1,2,4-trithiolane (No. 1687), (±)-3-(methylthio)heptanal (No. 1692), ethyl methyl disulfide (No. 1693), ethyl propyl trisulfide (No. 1695), methyl isopentyl disulfide (No. 1696), butyl ethyl disulfide (No. 1698), allyl propyl disulfide (No. 1700) and bis(1-mercaptopropyl)sulfide (No. 1709), have assay values of <95%. Information on the safety of the secondary components of these 14 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 of diisopentyl thiomalate, diisopentyl thiotartronate, is expected to share the same metabolic fate as the primary substance. The secondary components of *trans*-l-mercapto-*p*-menthan-3-one, piperitone (No. 435) and α-terpineol (No. 366), are expected to undergo rapid absorption, distribution, metabolism and excretion and were evaluated at previous meetings (Annex 1, references *137* and *166*). The secondary component of (±)-2,8-epithio-*cis*-*p*-menthane, D-limonene (No. 1326), is expected to undergo rapid absorption, distribution, metabolism and excretion and was evaluated at a previous meeting (Annex 1, reference *173*). The secondary component of (±)-3-(methylthio)heptanal, *trans*-2-heptenal (No. 1360), is

expected to undergo rapid absorption, distribution, metabolism and excretion and was evaluated at a previous meeting (Annex 1, reference *173*). The secondary components of ethyl methyl disulfide, diethyl disulfide and dimethyl disulfide (No. 564), are expected to share the same metabolic fate as ethyl methyl disulfide. Dimethyl disulfide was evaluated at a previous meeting (Annex 1, reference *160*). The secondary components of ethyl propyl trisulfide, diethyl trisulfide and dipropyl trisulfide (No. 585), are expected to share the same metabolic fate as ethyl propyl trisulfide. Dipropyl trisulfide was evaluated at a previous meeting (Annex 1, reference *149*). The secondary components of methyl isopentyl disulfide, dimethyl disulfide (No. 564) and diisopentyl disulfide, are expected to share the same metabolic fate as methyl isopropyl disulfide. Dimethyl disulfide was evaluated at a previous meeting (Annex 1, reference *160*). The secondary components of butyl ethyl disulfide, diethyl disulfide and dibutyl disulfide, are expected to share the same metabolic fate as butyl ethyl disulfide. The secondary components of allyl propyl disulfide, allyl propylsulfide and dipropylsulfide, are expected to share the same metabolic fate as allyl propyl disulfide. The secondary component of bis(1-mercaptopropyl)sulfide, 3,5-diethyl-1,2,4-trithiolane (No. 1686), was evaluated at the present meeting and is predicted to undergo reduction to free dithiol and *S*-oxidation of the cyclic thioether with subsequent excretion. Owing to their malodorous nature, the following flavouring agents are available in solution: ethane-1,1-dithiol in 1% ethanol (No. 41), 4-mercapto-2-pentanone in 1% acetoin (No. 405), 2,4,6-trithiaheptane in 10% triacetin (No. 920) and the mixture of 3,6-diethyl-1,2,4,5-tetrathiane and 3,5-diethyl-1,2,4-trithiolane in 1% vegetable oil. The first three solvents have been evaluated at previous meetings (Annex 1, references *122*, *137* and *160*), and vegetable oil is a common component of traditional foods. None of the secondary components is considered to present a safety concern at current levels of intake as flavouring agents.

Conclusion

In the previous evaluations of substances in this group, studies of acute toxicity, short-term toxicity (14 days to 14 weeks), long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None raised safety concerns. The toxicity data available for this evaluation were supported by those from the previous evaluations.

The Committee concluded that these 51 flavouring agents, which are additions to the group of simple aliphatic and aromatic sulfides and thiols evaluated previously, would not give rise to safety concerns at the currently estimated levels of intake.

An addendum to the toxicological monograph was prepared.

4.1.4 *Aliphatic acyclic diols, triols and related substances: additional compounds*

The Committee was requested to evaluate 13 members of a group of aliphatic acyclic diols, triols and related substances. The Committee noted that five substances (listed as Nos 1720, 1721 and 1723–1725) submitted for consideration as members of this group are various fatty acid esters of glycerol and propylene glycol. These substances were previously evaluated by the Committee as emulsifying agents. These substances have specifications and have been allocated ADIs. Although the use of these substances as flavouring agents would not be anticipated to cause a safety concern, the Committee questioned whether these substances have flavouring properties. The Committee decided not to evaluate them according to the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). In addition, the Committee questioned the flavouring function of lactylated fatty acid esters of glycerol and propylene glycol (listed as No. 1722), for which an ADI and specifications are not available, and decided not to evaluate No. 1722 as a flavouring agent using the Procedure.

From the proposed group, the Committee evaluated seven aliphatic acyclic diols and related substances, including three diol acetals (Nos 1711, 1712 and 1715) and four mono- and dihydroxy derivatives (Nos 1716–1719), using the Procedure.

The Committee previously evaluated 31 other members of this chemical group of flavouring agents at its fifty-seventh meeting (Annex 1, reference *154*). The findings in that report were considered in the present evaluation. The Committee concluded that 17 of the 31 substances in that group were of no safety concern at the estimated current levels of intake. The 17 flavouring agents included two dioxolane derivatives (Nos 838 and 839). The evaluation of the remaining 14 substances could not be finalized, as the Committee requested further data to determine whether these substances are currently used as flavouring agents.

At the subsequent meeting, data on the use of these compounds as flavours were provided to the Committee by the flavour industry (Annex 1, reference *160*). With the exception of glycerol (No. 909) and propylene glycol (No. 925), for which the Committee considered the data provided to be inadequate to substantiate the use of these substances as flavouring agents, the Committee finalized the evaluations of all other agents in this group and concluded that 12 additional flavouring agents were of no safety concern at the estimated current levels of intake as flavouring agents.

At its seventh meeting, the Committee evaluated propylene glycol (No. 925) and assigned an ADI of 0–20 mg/kg bw (Annex 1, reference *7*). At the

seventeenth meeting, the ADI for propylene glycol (No. 925) was established at 0–25 mg/kg bw (Annex 1, reference *32*). Glycerol, a metabolite of propylene glycol, was evaluated at the twentieth meeting, at which an ADI "not specified" was assigned (Annex 1, reference *41*). In addition to its evaluation for use as a flavouring agent using the Procedure, lactic acid (No. 930), also a metabolite of propylene glycol, was evaluated by the Committee at its seventeenth meeting and allocated an ADI "not limited" (Annex 1, reference *32*).

Three of the seven flavouring agents (Nos 1717–1719) being evaluated at the current meeting have been reported to occur as natural components of food. They have been detected in coffee, mushrooms, pineapple, apple cider and port wine. Consumption ratios (the ratios of their consumption from natural food sources to their use as flavouring agents) were not calculated because no quantitative data were available.

Assessment of dietary exposure

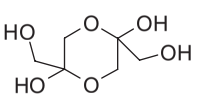
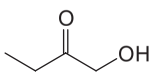
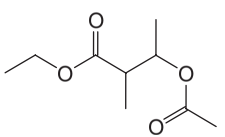
The total annual volumes of production of the seven aliphatic acyclic diols and related flavouring agents being evaluated at this meeting are approximately 1445 kg in Europe, 53 kg in the USA and 887 kg in Japan. Greater than 87% of the annual volume in Europe is accounted for by 2,4-dimethyl-1,3-dioxolane (No. 1711). Greater than 94% of the annual volume in Japan is accounted for by 2,4-dimethyl-1,3-dioxolane (No. 1711), ethyl 2,4-dimethyl-1,3-dioxolane-2-acetate (No. 1715) and dihydroxyacetone dimer (No. 1716). In the USA, dihydroxyacetone dimer (No. 1716) comprises the entire annual volume. The daily per capita intake of each agent is reported in Table 6.

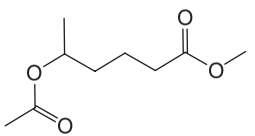
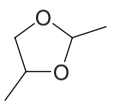
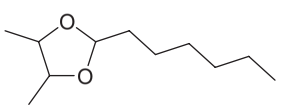
Absorption, distribution, metabolism and excretion

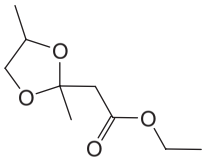
Acetals, ketals and esters are hydrolysed to their component alcohols and aldehydes, ketones and acids, respectively. The metabolism of two of the dioxolanes (Nos 1711 and 1715) yields propylene glycol. Propylene glycol is metabolized to endogenous glycerol, lactic acid, pyruvic acid and simple aliphatic alcohols, aldehydes and acids that are completely metabolized to carbon dioxide and water. In the glycolytic pathway, glycerol is converted to glyceraldehyde-3-phosphate and enters the glycolytic pathway to eventually yield pyruvic acid. Multiple pathways are available to the other flavouring agents (Nos 1712 and 1716–1719) in this group. These include phosphorylation and conjugation with glucuronic acid.

Table 6

Summary of the results of safety evaluations of aliphatic acyclic diols, triols and related substances 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?	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I							
Dihydroxyacetone dimer	1716	62147-49-3 	No Europe: 0.01 USA: 6 Japan: 74	NR	NR	See note 1	No safety concern
1-Hydroxy-2-butanone	1717	5077-67-8 	No Europe: 0.01 USA: ND Japan: 0.08	NR	NR	See note 2	No safety concern
Ethyl 3-acetoxy-2-methylbutyrate	1718	139564-43-5 	No Europe: 0.01 USA: ND Japan: ND	NR	NR	See note 3	No safety concern

Methyl 5-acetoxyhexanoate	1719	35234-22-1 	No Europe: 0.01 USA: ND Japan: ND	NR	NR	See note 3	No safety concern
Structural class III							
2,4-Dimethyl-1,3-dioxolane	1711	3390-12-3 	Yes Europe: 135 USA: ND Japan: 122	No	Yes. The NOEL of 2500 mg/kg bw per day for the non-endogenous metabolite propylene glycol (No. 925) is >1 million times the estimated intake of 2,4-dimethyl-1,3-dioxolane when used as a flavouring agent.	See note 4	No safety concern
2-Hexyl-4,5-dimethyl-1,3-dioxolane	1712	6454-22-4 	No Europe: 0.01 USA: ND Japan: ND	NR	NR	See note 4	No safety concern

cis- and *trans*-Ethyl 2,4-dimethyl-1,3-dioxolane-2-acetate	1715	6290-17-1 	No Europe: 19 USA: ND Japan: 38	NR	NR	See note 5	No safety concern

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.

[a] Thirty-one flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *155*).

[b] *Step 1:* Four of the flavouring agents in this group are in structural class I, and three are in structural class III.

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

[d] The threshold for human intakes for structural classes I and III are 1800 and 90 µg/person per day, respectively. All intake values are expressed in µg/day.

Notes to Table 6:

1. Dihydroxyacetone dimer is readily converted to dihydroxyacetone phosphate, which participates in several metabolic pathways.
2. Detoxicated via conjugation with glucuronic acid and subsequent elimination in the urine.
3. Hydrolysed to the corresponding alcohols and acid, which then enter known pathways of metabolism.
4. Detoxicated by hydrolysis to the corresponding aldehyde and aliphatic glycol, which are both completely metabolized by known pathways.
5. Detoxicated by hydrolysis to the corresponding ketone and aliphatic glycol, which are both completely metabolized by known pathways.

Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure to the above-mentioned seven flavouring agents, the Committee assigned four flavouring agents to structural class I (Nos 1716–1719) and three to structural class III (Nos 1711, 1712 and 1715).

Step 2. All of the flavouring agents in this group are expected to be metabolized to innocuous products. The evaluation of all 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). For two flavouring agents (Nos 1712 and 1715) in structural class III, the estimated daily per capita intakes are below the threshold of concern (i.e. 90 μg/person per day for class III). According to the Procedure, the safety of these six flavouring agents raises no concern when they are used at their current estimated levels of intake. For one flavouring agent (No. 1711) in class III, the estimated daily per capita intake exceeds the threshold (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of this flavouring agent proceeded to step A4.

Step A4. Although one metabolite of No. 1711 is an endogenous substance (acetic acid), the other metabolite (propylene glycol) is not. Accordingly, the evaluation of this flavouring agent proceeded to step A5.

Step A5. The NOEL of 2500 mg/kg bw per day for propylene glycol (No. 925) from a 2-year study of toxicity in rats is >1 million times the estimated intake of the parent compound 2,4-dimethyl-1,3-dioxolane (No. 1711) from its use as a flavouring agent. The Committee therefore concluded that 2,4-dimethyl-1,3-dioxolane would not pose a safety concern at the currently estimated level of intake.

Table 6 summarizes the stepwise evaluation of these seven flavouring agents.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are predicted to be metabolized by hydrolysis, pathways of intermediate metabolism and/or conjugation. These pathways have a high capacity and would not be saturated, even if all flavouring agents were consumed at the same time. A number of the flavouring agents in this group that have been evaluated at this meeting and at the fifty-seventh meeting are predicted to be hydrolysed to common intermediary metabolites, such as lactate and pyruvate. The Committee concluded that combined intakes would not raise safety concerns.

Consideration of secondary components

One flavouring agent in this group, 1-hydroxy-2-butanone (No. 1717), has a minimum assay value of <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 of 1-hydroxy-2-butanone is acetoin (No. 405), which is expected to share the same metabolic fate as 1-hydroxy-2-butanone. Acetoin (No. 405) was evaluated by the Committee for its use as a flavouring agent at the fifty-first meeting (Annex 1, reference *137*) and considered not to present a safety concern at current levels of intake.

Conclusion

In the previous evaluation of flavouring agents in this group of aliphatic acyclic diols, triols and related substances, studies of acute toxicity, short-term toxicity, long-term toxicity and carcinogenicity and genotoxicity were available. None raised safety concerns. The toxicity data available for this evaluation were supported by those from previous evaluations at the fifty-seventh meeting.

The Committee concluded that these seven flavouring agents, which are additions to the group evaluated previously, would not give rise to safety concerns at the currently estimated levels of intake. Six other substances proposed for evaluation in this group were not evaluated using the Procedure, as the Committee questioned whether they had flavouring properties.

An addendum to the toxicological monograph was prepared.

4.1.5 *Aliphatic acetals: additional compounds*

The Committee evaluated 24 aliphatic acetals as flavouring agents by the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these agents has previously been evaluated.

The Committee previously evaluated 10 other members of this chemical group of flavouring agents at its fifty-seventh meeting (Annex 1, reference *154*). The findings presented in that report were considered in the present evaluation. All 10 substances in that group were concluded to be of no safety concern based on currently estimated levels of intake.

Six of the 24 flavouring agents in this group are natural components of foods (Nos 1726, 1729, 1730, 1732, 1734 and 1748). They have been detected primarily in alcoholic beverages, including white and red wine, brandy, rum, cognac and sherry. They have also been detected in cider, baked potato and pork (*27*). Because no quantitative data were provided, consumption ratios

(the ratios of their consumption from natural food sources to their use as flavouring agents) were not calculated.

Under aqueous acidic conditions, as may occur in foods and after consumption in the gastric fluids, acetals hydrolyse to yield alcohols and aldehydes (*40*, *41*). In some instances, hydrolysis to an acid has also been reported (*42*). The component alcohol and aldehyde hydrolysis products of acetals are ubiquitous in nature and are naturally occurring components of a wide variety of traditional foods. Low molecular weight alcohols have been detected in almost every known fruit and vegetable. Branched-chain primary alcohols and aldehydes have been detected in foods such as cheese, fruits, vinegar and alcoholic beverages (*27*). In addition, many low molecular weight alcohols (e.g. ethanol), aldehydes (e.g. acetaldehyde) and acids are endogenous in humans (*43*). The Committee has previously evaluated many of the component alcohols, aldehydes and acids (Annex 1, references *122*, *131*, *137*, *154* and *166*).

Assessment of dietary exposure

The total annual volume of production of the 24 aliphatic acetals is approximately 16 kg in Europe and 266 kg in Japan (*3*, *4*). The daily per capita intake of each agent is reported in Table 7. Annual volumes of production of this group of flavouring agents are summarized in Table 8.

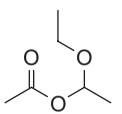
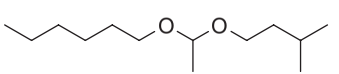
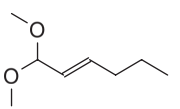
Absorption, distribution, metabolism, elimination and toxicity

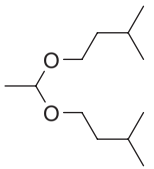
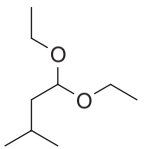
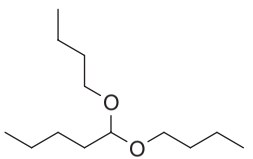
In general, aliphatic acetals undergo hydrolysis to their component aldehydes, alcohols and acids (*40*, *41*, *44*, *45*, *46*, *47*). Following hydrolysis, the component alcohols (*43*, *48*) and aldehydes (*49*) are readily absorbed through the gastrointestinal tract, rapidly eliminated from the blood and subsequently metabolized via the fatty acid pathway or the citric acid cycle. Additionally, 1-acetoxy-1-ethoxyethane (No. 1726) shows rapid hydrolysis to acetaldehyde, acetic acid and ethanol in aqueous solution at pH 2.5 (*42*).

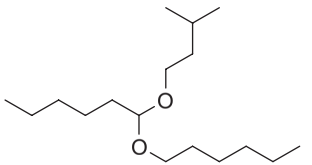
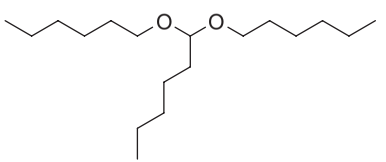
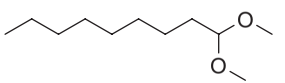
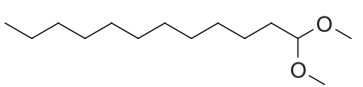
The toxicological data presented at the fifty-seventh meeting of JECFA provided the foundation for the evaluation of these 24 flavouring agents. LD_{50} values of 750 mg/kg bw or greater were reported for seven of the acetals and a number of their aldehyde, alcohol or acid metabolites. The LD_{50} of 1700 mg/kg bw in male rats for 1,1-dimethoxy-*trans*-2-hexene (No. 1728) is comparable (*50*). The results of a number of short-term and long-term studies (toxicity, carcinogenicity and reproductive toxicity) on a number of the aldehyde and/or alcohol metabolites of these 10 acetals revealed NOEL values in the range of 10–200 mg/kg bw per day or greater. The results of genotoxicity studies demonstrated that these acetals and their metabolites were not genotoxic in vivo.

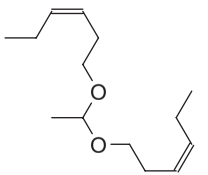
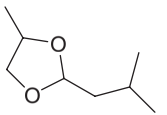
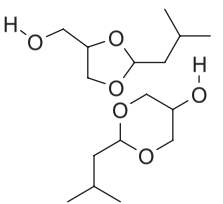
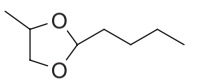
Table 7

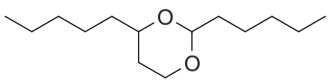
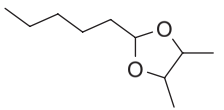
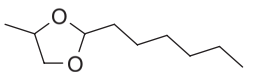
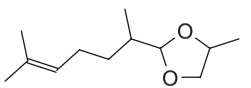
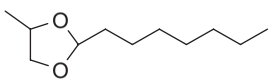
Summary of the results of safety evaluations of aliphatic acetals used as flavouring agents[a,b,c]

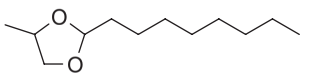
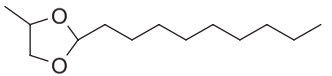
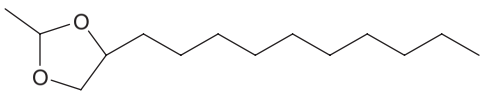
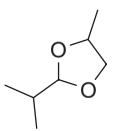
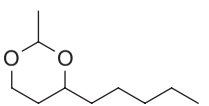
Flavouring agent	No.	CAS No. and structure	*Step A3*[d] Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I					
(±)-1-Acetoxy-1-ethoxyethane	1726	1608-72-6 	No Europe: 2 USA: ND Japan: ND	See note 1	No safety concern
Acetaldehyde hexyl isoamyl acetal	1727	233665-90-2 	No Europe: ND USA: ND Japan: 4	See note 2	No safety concern
1,1-Dimethoxy-*trans*-2-hexene	1728	18318-83-7 	No Europe: 0.01 USA: ND Japan: 0.03	See note 3	No safety concern

Acetaldehyde diisoamyl acetal	1729	13002-09-0 	No Europe: 0.01 USA: ND Japan: 0.6	See note 4	No safety concern
Isovaleraldehyde diethyl acetal	1730	3842-03-3 	No Europe: 0.09 USA: ND Japan: 16	See note 5	No safety concern
Valeraldehyde dibutyl acetal	1731	13112-65-7 	No Europe: ND USA: ND Japan: 2	See note 6	No safety concern

Hexanal hexyl isoamyl acetal	1735	896447-13-5 	No Europe: ND USA: ND Japan: 5	See note 7	No safety concern
Hexanal dihexyl acetal	1738	33673-65-3 	No Europe: ND USA: ND Japan: 5	See note 8	No safety concern
Nonanal dimethyl acetal	1742	18824-63-0 	No Europe: ND USA: ND Japan: 2	See note 9	No safety concern
Dodecanal dimethyl acetal	1746	14620-52-1 	No Europe: ND USA: ND Japan: 0.7	See note 10	No safety concern

Acetaldehyde di-*cis*-3-hexenyl acetal	1747	63449-64-9 	No Europe: ND USA: ND Japan: 0.6	See note 11	No safety concern
Structural class III					
Isovaleraldehyde propyleneglycol acetal	1732	18433-93-7 	No Europe: ND USA: ND Japan: 11	See note 12	No safety concern
Isovaleraldehyde glyceryl acetal	1733	54355-74-7 	No Europe: ND USA: ND Japan: 0.8	See note 13	No safety concern
Valeraldehyde propyleneglycol acetal	1734	74094-60-3 	No Europe: ND USA: ND Japan: 3	See note 14	No safety concern

Hexanal octane-1,3-diol acetal	1736	202188-46-3 	No Europe: ND USA: ND Japan: 0.003	See note 15	No safety concern
Hexanal butane-2,3-diol acetal	1737	155639-75-1 	No Europe: ND USA: ND Japan: 0.003	See note 16	No safety concern
Heptanal propyleneglycol acetal	1739	4351-10-4 	No Europe: ND USA: ND Japan: 0.3	See note 17	No safety concern
2,6-Dimethyl-5-heptenal propyleneglycol acetal	1740	74094-63-6 	No Europe: ND USA: ND Japan: 0.4	See note 18	No safety concern
Octanal propyleneglycol acetal	1741	74094-61-4 	No Europe: ND USA: ND Japan: 3	See note 19	No safety concern

Nonanal propyleneglycol acetal	1743	68391-39-9 	No Europe: ND USA: ND Japan: 0.4	See note 20	No safety concern
Decanal propyleneglycol acetal	1744	5421-12-5 	No Europe: ND USA: ND Japan: 13	See note 21	No safety concern
Undecanal propyleneglycol acetal	1745	74094-62-5 	No Europe: ND USA: ND Japan: 0.1	See note 22	No safety concern
Isobutanal propyleneglycol acetal	1748	67879-60-1 	No Europe: ND USA: ND Japan: 3	See note 23	No safety concern
Acetaldehyde 1,3-octanediol acetal	1749	202188-43-0 	No Europe: ND USA: ND Japan: 0.003	See note 24	No safety concern

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

[a] Ten flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *155*).

[b] *Step 1*: Eleven flavouring agents in this group are in structural class I, and 13 flavouring agents are in structural class III.

[c] S*tep 2*: All of 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 III are 1800 and 90 µg/person per day, respectively. All intake values are expressed in µg/day.

Notes to Table 7:

1. Predicted to be metabolized to ethanol (No. 41), acetaldehyde (No. 80) and acetic acid (No. 81).
2. Predicted to be metabolized to hexanol (No. 91), isoamyl alcohol (No. 52) and acetaldehyde (No. 80).
3. Predicted to be metabolized to methanol and 2-hexenal (No. 1353).
4. Predicted to be metabolized to isoamyl alcohol (No. 52) and acetaldehyde (No. 80).
5. Predicted to be metabolized to ethanol (No. 41) and 3-methylbutyraldehyde (No. 258).
6. Predicted to be metabolized to butanol (No. 85) and valeraldehyde (No. 89).
7. Predicted to be metabolized to hexanol (No. 91), isoamyl alcohol (No. 52) and hexanal (No. 92).
8. Predicted to be metabolized to hexanol (No. 91) and hexanal (No. 92).
9. Predicted to be metabolized to methanol and nonanal (No. 101).
10. Predicted to be metabolized to methanol and dodecanal (No. 110).
11. Predicted to be metabolized to *cis*-3-hexenol (No. 315) and acetaldehyde (No. 80).
12. Predicted to be metabolized to propylene glycol (No. 925) and 3-methylbutyraldehyde (No. 258).
13. Predicted to be metabolized to glycerol (No. 909) and 3-methylbutyraldehyde (No. 258).
14. Predicted to be metabolized to propylene glycol (No. 925) and valeraldehyde (No. 89).
15. Predicted to be metabolized to 1,3-octanediol and hexanal (No. 92).
16. Predicted to be metabolized to 2,3-butanediol and hexanal (No. 92).
17. Predicted to be metabolized to propylene glycol (No. 925) and heptanal (No. 95).
18. Predicted to be metabolized to propylene glycol (No. 925) and 2,6-dimethyl-5-heptenal (No. 349).
19. Predicted to be metabolized to propylene glycol (No. 925) and octanal (No. 98).
20. Predicted to be metabolized to propylene glycol (No. 925) and nonanal (No. 101).
21. Predicted to be metabolized to propylene glycol (No. 925) and decanal (No. 104).
22. Predicted to be metabolized to propylene glycol (No. 925) and undecanal (No. 107).
23. Predicted to be metabolized to propylene glycol (No. 925) and isobutyraldehyde (No. 252).
24. Predicted to be metabolized to 1,3-octanediol and acetaldehyde (No. 80).

Table 8

Annual volumes of production of aliphatic acetals used as flavouring agents in Europe, the USA and Japan

Flavouring agent (No.)	Most recent annual volume (kg)[a]	Intake[b]		Intake of alcohol equivalents (μg/kg bw per day)[c]	Intake of aldehyde equivalents (μg/kg bw per day)[d]
		μg/day	μg/kg bw per day		
(±)-1-Acetoxy-1-ethoxyethane (1726)					
Europe	15	2	0.03	0.01	0.01
USA	ND	NA	NA		
Japan	ND	NA	NA		
Acetaldehyde hexyl isoamyl acetal (1727)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	14	4	0.06	0.05	0.01
1,1-Dimethoxy-*trans*-2-hexene (1728)					
Europe	0.1	0.01	0.0002	0.000 09	0.0001
USA	ND	NA	NA		
Japan	0.1	0.03	0.0005	0.0002	0.0003
Acetaldehyde diisoamyl acetal (1729)					
Europe	0.1	0.01	0.0002	0.0002	0.000 04
USA	ND	NA	NA		
Japan	2	0.6	0.009	0.008	0.002
Isovaleraldehyde diethyl acetal (1730)					
Europe	0.9	0.09	0.002	0.001	0.001
USA	ND	NA	NA		
Japan	60	16	0.3	0.2	0.2
Valeraldehyde dibutyl acetal (1731)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	7	2	0.03	0.02	0.01
Isovaleraldehyde propyleneglycol acetal (1732)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	40	11	0.2	0.1	0.1

Isovaleraldehyde glyceryl acetal (1733)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	3	0.8	0.01	0.006	0.005
Valeraldehyde propyleneglycol acetal (1734)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	11	3	0.05	0.03	0.03
Hexanal hexyl isoamyl acetal (1735)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	19	5	0.08	0.06	0.03
Hexanal octane-1,3-diol acetal (1736)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	0.1	0.03	0.0004	0.0002	0.0002
Hexanal butane-2,3-diol acetal (1737)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	0.1	0.03	0.0004	0.0002	0.0002
Hexanal dihexyl acetal (1738)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	20	5	0.09	0.06	0.03
Heptanal propyleneglycol acetal (1739)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	1	0.3	0.005	0.002	0.003
2,6-Dimethyl-5-heptenal propyleneglycol acetal (1740)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	2	0.4	0.007	0.003	0.005

Octanal propyleneglycol acetal (1741)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	10	3	0.04	0.02	0.03
Nonanal dimethyl acetal (1742)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	8	2	0.03	0.01	0.02
Nonanal propyleneglycol acetal (1743)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	2	0.4	0.007	0.003	0.005
Decanal propyleneglycol acetal (1744)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	51	13	0.2	0.07	0.2
Undecanal propyleneglycol acetal (1745)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	0.5	0.1	0.002	0.0007	0.002
Dodecanal dimethyl acetal (1746)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	3	0.7	0.01	0.003	0.008
Acetaldehyde di-*cis*-3-hexenyl acetal (1747)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	2	0.6	0.01	0.009	0.002
Isobutanal propyleneglycol acetal (1748)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	11	3	0.05	0.03	0.03

Acetaldehyde 1,3-octanediol acetal (1749)					
Europe	ND	NA	NA		
USA	ND	NA	NA		
Japan	0.1	0.03	0.0004	0.0003	0.0001
Total					
Europe	16				
USA	ND				
Japan	266				

ND, no data reported; NA, not applicable.

[a] From references *3*, *4*, *5*. Total poundage values of <1 kg reported in the surveys (*3*, *4*, *5*) 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; where correction factor = 0.8 for surveys in Europe, USA and Japan, representing the assumption that only 80% of the annual flavour volume was reported in the poundage surveys (*3*, *4*, *5*).
Intake (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur as a result of rounding.

[c] Calculated as follows: (molecular weight of alcohol/molecular weight of acetal) × daily per capita intake ("consumers only") of acetal. Slight variations may occur as a result of rounding.

[d] Calculated as follows: (molecular weight of aldehyde/molecular weight of acetal) × daily per capita intake ("consumers only") of acetal. Slight variations may occur as a result of rounding.

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 11 flavouring agents to structural class I and 13 to structural class III (*38*).

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

Step A3. The estimated daily per capita intakes of the 24 flavouring agents in this group are below the threshold of concern for class I (i.e. 1800 μg/person per day) and for class III (i.e. 90 μg/person per day). According to the Procedure, the safety of these 24 flavouring agents raises no concern when they are used at their currently estimated levels of intake.

Table 7 summarizes the evaluations of the 24 acetals included in this group of flavouring agents.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are predicted to be metabolized by hydrolysis and/or oxidative metabolism of alcohol and aldehyde groups and alkyl side-chains and/or by pathways of intermediary metabolism. These pathways have a high capacity and would not be saturated, even if all flavouring agents were consumed at the same time. A number of the substances in this group that have been evaluated at this meeting and at the fifty-seventh meeting are predicted to be hydrolysed to common aldehyde and alcohol metabolites. Common aldehyde metabolites (and their alcohol precursors) are acetaldehyde (Nos 940, 941, 943, 1726, 1727, 1729, 1747 and 1749), valeraldehyde or isovaleraldehyde (Nos 1730–1734), hexanal (Nos 1735–1738), heptanal (Nos 947 and 1739), octanal (Nos 942 and 1741), nonanal (Nos 1742 and 1743) and decanal (Nos 945, 1744 and 1746), all of which are in class I. The resulting total intake of common metabolites arising from combined intakes of these substances is below the threshold for class I. The Committee concluded that under the conditions of use as flavouring agents, the combined intake 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

No members of this group have an assay value of <95%.

Conclusion

In the previous evaluations of substances in this group, studies of acute toxicity, short-term toxicity (28–150 days), long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None raised safety concerns. The data available for this evaluation supported those from the previous evaluations.

The Committee concluded that these 24 flavouring agents, which are additions to the group of aliphatic acetals evaluated previously, would not give rise to safety concerns at the currently estimated levels of intake.

An addendum to the toxicological monograph was not prepared.

4.1.6 *Sulfur-containing heterocyclic compounds: additional compounds*

The Committee evaluated a group of 17 flavouring agents comprising sulfur-containing heterocyclic compounds using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). The group was composed of both five- and six-member sulfur-containing aromatic and non-aromatic heterocyclic compounds, including three thiophene (Nos 1750,

1764 and 1765), eight thiazole (Nos 1751, 1752, 1753, 1754, 1755, 1756, 1757 and 1758), four thiazoline (Nos 1759, 1760, 1761 and 1762), one thiazine (No. 1766) and one dithiazine (No. 1763) derivative. The Committee has not evaluated these flavouring agents previously.

The Committee previously evaluated 30 other members of this chemical group of flavouring agents at its fifty-ninth meeting (Annex 1, reference *160*). The findings presented in that report were considered in the present evaluation. For all 30 substances, the Committee concluded that there were no safety concerns at the currently estimated levels of intake.

Three of the 17 flavouring agents (Nos 1758, 1759 and 1764) in this group have been reported to occur naturally in coffee, black tea, barley, chicken, turkey, guinea hen, beef, mushrooms, trassi, American cranberry and sweet corn (*27*). No quantitative data on the natural levels in food were available, and therefore consumption ratios (the ratios of their consumption from natural food sources to their use as flavouring agents) were not calculated.

Assessment of dietary exposure

The total annual volume of production of this group of sulfur-containing heterocyclic compounds is approximately 86 kg in Europe, 2 kg in the USA and 1145 kg in Japan. Approximately 88% of the total annual volume of production in Europe is accounted for by 2-acetyl-2-thiazoline (No. 1759). The daily per capita intake of 2-acetyl-2-thiazoline (No. 1759) is 8 μg/kg in Europe and 4 μg/kg in Japan. Approximately 94% of the total annual volume of production in Japan is accounted for by four flavouring agents: 2-(4-methyl-5-thiazolyl)ethyl butanoate (No. 1753), 2-(4-methyl-5-thiazolyl)ethyl isobutyrate (No. 1754), 2-(4-methyl-5-thiazolyl)ethyl octanoate (No. 1756) and 2-(4-methyl-5-thiazolyl)ethyl decanoate (No. 1757). The daily per capita intake of each flavouring agent is reported in Table 9.

Absorption, distribution, metabolism and elimination

The metabolism of sulfur-containing heterocyclic compounds was previously described in the report of the fifty-ninth meeting. Thiazole and its derivatives are metabolized primarily by side-chain oxidation or oxidation of the ring sulfur or nitrogen atoms; however, other routes of metabolism, involving ring cleavage, are possible. Seven of the thiazole derivatives in this group are 2-thiazolylethyl esters (Nos 1751–1757) and are hydrolysed to form 4-methyl-5-thiazoleethanol (No. 1031), a normal metabolite of vitamin B_1 (thiamine), which is further oxidized and excreted free or as a glutathione conjugate.

Thiazoline derivatives (Nos 1759–1762), being cyclic sulfides, are metabolized primarily by *S*-oxidation to yield corresponding sulfoxides and sulfones.

Thiophene derivatives are subject to *S*-oxidation followed by conjugation with glutathione; however, other routes of metabolism, involving ring cleavage, are also possible. The resulting mercapturic acid derivative is eliminated in the urine.

Thiazine and dithiazine derivatives are expected to be metabolized primarily via side-chain oxidation and ring *S*- and *N*-oxidation.

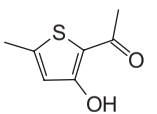
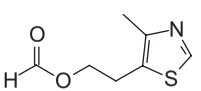
Application of the Procedure for the Safety Evaluation of Flavouring Agents

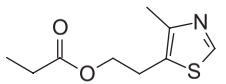
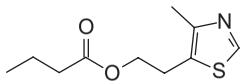
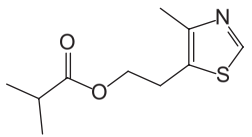
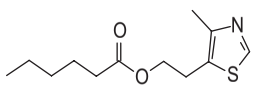
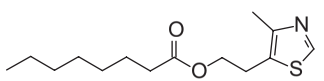
Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned flavouring agents, the Committee assigned 13 flavouring agents (Nos 1750–1760, 1764 and 1766) to structural class II and 4 flavouring agents (Nos 1761–1763 and 1765) to structural class III.

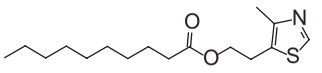
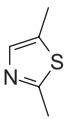
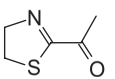
Step 2. The seven 2-thiazolylethyl esters (Nos 1751–1757) are expected to be readily hydrolysed to the thiamine metabolite, 2-(4-methyl-5-thiazolyl) ethanol (No. 1031), which in turn is metabolized to an innocuous product. The evaluation of the seven esters therefore proceeded via the A-side of the Procedure. For the remaining 10 flavouring agents in this group (Nos 1750 and 1758–1766), the data were insufficient to allow conclusions about their probable metabolic fate. Therefore, for these flavouring agents, the evaluation proceeded via the B-side of the Procedure.

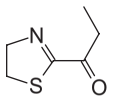
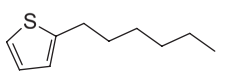
Table 9

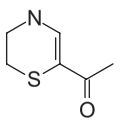
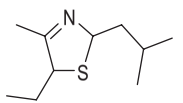
Summary of the results of safety evaluations of sulfur-containing heterocyclic compounds used as flavouring agents[a,b,c]

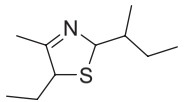
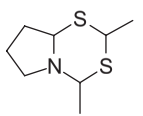
Flavouring agent	No.	CAS No. and structure	*Step 2* Predicted to be metabolized to innocuous products?	*Step A3/B3* Does intake exceed the threshold for human intake?[d]	*Step A4* Is the flavouring agent or are its metabolites endogenous?	*Step A5/B4* Adequate margin of safety for the flavouring agent or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class II								
1-(3-Hydroxy-5-methyl-2-thienyl)ethanone	1750	133860-42-1 	No—B-side	No Europe: 0.01 USA: ND Japan: ND	NR	Yes. The NOEL of 290 mg/kg bw per day for the related substance 2-thienyldisulfide (No. 1053) is >1 billion times the estimated intake of 1-(3-hydroxy-5-methyl-2-thienyl) ethanone when used as a flavouring agent.	See note 3	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl formate	1751	90731-56-9 	Yes—A-side	No Europe: ND USA: ND Japan: 2	NR	NR	See note 2	No safety concern

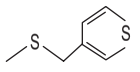
2-(4-Methyl-5-thiazolyl)ethyl propionate	1752	324742-96-3 	Yes—A-side	No Europe: ND USA: ND Japan: 10	NR	NR	See note 2	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl butanoate	1753	94159-31-6 	Yes—A-side	No Europe: ND USA: ND Japan: 78	NR	NR	See note 2	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl isobutyrate	1754	324742-95-2 	Yes—A-side	No Europe: ND USA: ND Japan: 31	NR	NR	See note 2	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl hexanoate	1755	94159-32-7 	Yes—A-side	No Europe: ND USA: ND Japan: 4	NR	NR	See note 2	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl octanoate	1756	163266-17-9 	Yes—A-side	No Europe: ND USA: ND Japan: 144	NR	NR	See note 2	No safety concern

2-(4-Methyl-5-thiazolyl)ethyl decanoate	1757	101426-31-7 	Yes—A-side	No Europe: ND USA: ND Japan: 30	NR	NR	See note 2	No safety concern
2,5-Dimethylthiazole	1758	4175-66-0 	No—B-side	No Europe: 0.01 USA: ND Japan: 0.03	NR	Yes. The NOEL of 0.93 mg/kg bw per day for the related substance 2,4-dimethyl-5-vinylthiazole (No. 1039) is >2 million times the estimated intake of 2,5-dimethylthiazole when used as a flavouring agent.	See note 1	No safety concern
2-Acetyl-2-thiazoline	1759	29926-41-8 	No—B-side	No Europe: 8 USA: ND Japan: 4	NR	Yes. The NOEL of 1.8 mg/kg bw per day is 18 000 and 30 000 times the estimated daily intake of 2-acetyl-2-thiazoline when used as a flavouring agent in Europe and Japan, respectively.	See note 1	No safety concern

2-Propionyl-2-thiazoline	1760	29926-42-9 	No—B-side	No Europe: 0.01 USA: ND Japan: ND	NR	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) is 6 million times the estimated intake of 2-propionyl-2-thiazoline when used as a flavouring agent.	See note 1	No safety concern
2-Hexylthiophene	1764	18794-77-9 	No—B-side	No Europe: 1 USA: 0.1 Japan: ND	NR	Yes. The NOEL of 290 mg/kg bw per day for the related substance 2-thienyldisulfide (No. 1053) is >14 million times the estimated intake of 2-hexylthiophene when used as a flavouring agent.	See note 3	No safety concern

5-Acetyl-2,3-dihydro-1,4-thiazine	1766	164524-93-0 	No—B-side	No Europe: ND USA: 0.2 Japan: ND	NR	Yes. The NOEL of 11 mg/kg bw per day for the mixture of related substances 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3, 5-dithiazine (No. 1046) is >3 million times the estimated intake of 5-acetyl-2,3-dihydro-1,4-thiazine when used as a flavouring agent.	See note 1	No safety concern
Structural class III								
cis- and *trans*-5-Ethyl-4-methyl-2-(2-methyl-propyl)thiazoline	1761	83418-53-5 	No—B-side	No Europe: 0.01 USA: ND Japan: ND	NR	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) is 6 million times the estimated intake of *cis*- and *trans*-5-ethyl-4-methyl-2-(2-methylpropyl) thiazoline when used as a flavouring agent.	See note 1	No safety concern

cis- and *trans*-5-Ethyl-4-methyl-2-(1-methylpropyl)thiazoline	1762	83418-54-6 	No—B-side	No Europe: 0.01 USA: ND Japan: ND	NR	Yes. The NOEL of 1.2 mg/kg bw per day for the related substance 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) is 6 million times the estimated intake of *cis*- and *trans*-5-ethyl-4-methyl-2-(2-butyl) thiazoline when used as a flavouring agent.	See note 1	No safety concern
Pyrrolidino-[1,2*E*]-4H-2,4-dimethyl-1,3,5-dithiazine	1763	116505-60-3 	No—B-side	No Europe: 0.01 USA: ND Japan: ND	NR	Yes. The NOEL of 11 mg/kg bw per day for the mixture of related substances 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (No. 1046) is 55 million times the estimated intake of pyrrolidino-[1,2*E*]-4H-2,4-dimethyl-1,3,5-dithiazine when used as a flavouring agent.	See note 1	No safety concern

3-(Methylthio)-methylthiophene	1765	61675-72-7 	No—B-side	No Europe: 0.01 USA: ND Japan: ND	NR	Yes. The NOEL of 290 mg/kg bw per day for the related substance 2-thienyldisulfide (No. 1053) is >1 billion times the estimated intake of 3-(methylthio)-methylthiophene when used as a flavouring agent.	See note 3	No safety concern

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required for evaluation because consumption of the substance was determined to be of no safety concern at Step A3 of the Procedure.

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

[b] *Step 1*: Ten flavouring agents are in structural class II, and seven are in structural class III.

[c] *Step 2:* All of the agents in this group cannot be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes II and III are 540 and 90 µg/day, respectively. All intake values are expressed in µg/day.

Notes to Table 9:

1. Metabolized primarily by side-chain oxidation and/or ring *S*- or *N*-oxidation. The major metabolites are readily excreted in the urine either free or as glucuronide or sulfate conjugates.
2. Enzymatically cleaved to yield 4-methyl-5-thiazoleethanol (No. 1031) and 2-methyl-4-amino-5-hydroxymethylpyrimidine. The thiazole and pyrimidine fragments are further oxidized to yield 4-methylthiazole-4-acetic acid and the 5-pyrimidine carboxylic acid derivative, respectively, which, together with thiamine, are excreted in the urine. May also be converted to 2-methyl-4-amino-5-formylaminopyrimidine and thiamine acetic acid.
3. Metabolized primarily by side-chain and/or ring *S*-oxidation followed by glutathione conjugation and elimination in the urine.

Step A3. With regard to those seven flavouring agents (Nos 1751–1757) evaluated via the A-side of the Procedure, the estimated daily per capita intakes are below the threshold of concern for the structural class (i.e. 540 μg/person per day for class II). According to the Procedure, there are no safety concerns for these seven flavouring agents when used at the currently estimated levels of intake.

Step B3. With regard to those 10 flavouring agents evaluated via the B-side of the Procedure, the estimated daily per capita intakes of the 6 flavouring agents in structural class II (Nos 1750, 1758–1760, 1764 and 1766) are below the threshold of concern (i.e. 540 μg/person per day for class II). Similarly, the estimated daily per capita intakes for the 4 flavouring agents in structural class III (Nos 1761–1763 and 1765) are below the threshold of concern (i.e. 90 μg/person per day for class III). The evaluation of these 10 flavouring agents therefore proceeded to step B4.

Step B4. The NOEL for 2-thienyldisulfide (No. 1053) in a 90-day dietary study in rats was 290 mg/kg bw per day (Annex 1, reference *160*), and this NOEL is appropriate to evaluate the structurally related flavouring agents 1-(3-hydroxy-5-methyl-2-thienyl)ethanone (No. 1750), 3-(methylthio)methylthiophene (No. 1765) and 2-hexylthiophene (No. 1764). The NOEL is >1 billion times the estimated intake of 1-(3-hydroxy-5-methyl-2-thienyl) ethanone and 3-(methylthio)methylthiophene from their use as flavouring agents in Europe (0.0002 μg/kg bw per day). The NOEL is >14 million times the estimated intake of 2-hexylthiophene from its use as a flavouring agent in Europe (0.02 μg/kg bw per day).

The NOEL for 2,4-dimethyl-5-vinylthiazole (No. 1039) in a 90-day study in rats was 0.93 mg/kg bw per day (Annex 1, reference *160*), and this NOEL is appropriate to evaluate the structurally related flavouring agent 2,5-dimethylthiazole (No. 1758). The NOEL is >2 million times the estimated intake of 2,5-dimethylthiazole from its use as a flavouring agent in Japan (0.0004 μg/kg bw per day).

The NOEL for 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture) (No. 1046) in a 90-day study in rats was 11 mg/kg bw per day (Annex 1, reference *160*), and this NOEL is appropriate to evaluate the structurally related flavouring agents pyrrolidino-[1,2e]-4H-2,4-dimethyl-1,3,5-dithiazine (No. 1763) and 5-acetyl-2,3-dihydro-1,4-thiazine (No. 1766). The NOEL is 55 million times the estimated intake of pyrrolidino-[1,2e]-4H-2,4-dimethyl-1,3,5-dithiazine from its use as a flavouring agent in Europe (0.0002 μg/kg bw per day). The NOEL is >3 million times the estimated intake of 5-acetyl-2,3-dihydro-1,4-thiazine from its use as a flavouring agent in the USA (0.003 μg/kg bw per day).

For 2-acetyl-2-thiazoline (No. 1759), the NOEL of 1.8 mg/kg bw per day from a 90-day rat study that examined the toxicity of a cocktail of flavours provides a margin of safety of 18 000 and 30 000 in relation to the estimated levels of exposure from its use as a flavouring agent in Europe (0.1 µg/kg bw per day) and in Japan (0.06 µg/kg bw per day).

The NOEL for 2-(2-butyl)-4,5-dimethyl-3-thiazoline (No. 1059) in a 90-day study in male rats was 1.2 mg/kg bw per day (Annex 1, reference *160*), and this NOEL is appropriate to evaluate the structurally related flavouring agents 2-acetyl-2-thiazoline (No. 1759), 2-propionyl-2-thiazoline (No. 1760), *cis*- and *trans*-5-ethyl-4-methyl-2-(2-methylpropyl)thiazoline (No. 1761) and *cis*- and *trans*-5-ethyl-4-methyl-2-(1-methylpropyl)thiazoline (No. 1762). The NOEL is 12 000 times the estimated intake of 2-acetyl-2-thiazoline from its use as a flavouring agent in Europe (0.1 µg/kg bw per day). The NOEL is 6 million times the estimated intakes of 2-propionyl-2-thiazoline, *cis*- and *trans*-5-ethyl-4-methyl-2-(2-methylpropyl)thiazoline and *cis*-and *trans*-5-ethyl-4-methyl-2-(1-methylpropyl)thiazoline from their use as flavouring agents in Europe (0.0002 µg/kg bw per day).

The NOEL for 2-acetyl-2-thiazole (No. 1041) in a 28-day study in rats was 50 mg/kg bw per day (Annex 1, reference *160*), and this NOEL is also appropriate to evaluate the structurally related flavouring agents 2-acetyl-2-thiazoline (No. 1759) and 2-propionyl-2-thiazoline (No. 1760). The NOEL is 500 000 times the estimated intake of 2-acetyl-2-thiazoline and 250 million times the estimated intake of 2-propionyl-2-thiazoline from their use as flavouring agents.

Table 9 summarizes the evaluations of the 17 sulfur-containing heterocyclic flavouring agents in this group.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are predicted to be metabolized by hydrolysis, by oxidative metabolism of heterocyclic rings and/or alkyl side-chains and by conjugation with glucuronic acid and/or glutathione. These pathways have a high capacity and would not be saturated, even if all flavouring agents were consumed at the same time. A number of the substances in this group that have been evaluated at this meeting and at the fifty-ninth meeting are predicted to be metabolized to a common metabolite. 4-Methyl-5-thiazoleethanol (No. 1031) is a predicted metabolite of Nos 1751–1757, and the combined intake would be below the threshold for class II. The other substances in this group have diverse structures, with various potential sites of metabolism, and are not likely to be metabolized to common products. The Committee concluded that under the conditions of use as flavouring agents, the combined intake of these substances would not saturate the metabolic pathways and the combined intakes would not raise safety concerns.

Consideration of secondary components

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

Conclusion

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

The Committee concluded that these 17 flavouring agents, which are additions to the group of sulfur-containing heterocyclic compounds evaluated previously, would not give rise to safety concerns at the currently estimated levels of intake.

An addendum to the toxicological monograph was prepared.

4.1.7 *Aliphatic and aromatic amines and amides: additional compounds*

The Committee evaluated a group of 12 flavouring agents, including 1 aliphatic amine (No. 1771); 6 aliphatic amides (Nos 1772–1776 and 1779), 4 of which contain ethanolamine (Nos 1772–1775) and 2 of which contain ring structures (Nos 1776 and 1779); and 5 aromatic amides (Nos 1767–1770 and 1777), 3 of which contain oxalamide (Nos 1768–1770). 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 has been evaluated previously by the Committee.

The Committee previously evaluated 37 other members of this group of flavouring agents at its sixty-fifth meeting (Annex 1, reference *178*). The findings presented in that report were considered in the present evaluation. Thirty-six of the 37 substances in that group were concluded to be of no safety concern based on currently estimated levels of intake. However, for 27 of the 36 agents, the intakes were estimated based on anticipated annual volumes of production; as such, the evaluations were deemed conditional pending submission of use levels or poundage data prior to December 2007. One substance, acetamide (No. 1592), was considered inappropriate for use as a flavouring agent or for food additive purposes, based on the available data that indicated that it is clearly carcinogenic in mice and rats. Therefore, the Committee did not evaluate this substance according to the Procedure.

Four of the 12 flavouring agents in this current group (Nos 1771, 1772, 1774 and 1777) have been reported to occur naturally in foods. They have been detected in white wine, spinach, potatoes, sweet potatoes, yams, kale, brown rice, brown rice germ, brown rice sprouts, barley, barley sprouts, beans, bean

sprouts, corn, oatmeal, squash, carrot, onion, chestnut, apple, shiitake mushrooms, green laver, lactobacilli and broccoli. No quantitative data on the natural levels in food were available, and therefore consumption ratios (the ratios of their consumption from natural food sources to their use as flavouring agents) were not calculated.

Assessment of dietary exposure

The total annual volume of production of this group of 12 aliphatic and aromatic amines and amides is approximately 785 kg in the USA and 0.4 kg in Europe. There were no reported uses of these flavouring agents in Japan. In the USA, greater than 91% of the total production volume is accounted for by *N*-gluconyl ethanolamine (No. 1772), *N*-lactoyl ethanolamine (No. 1774), *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide (No. 1776) and *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide (No. 1779). The estimated per capita intakes in the USA of *N*-gluconyl ethanolamine, *N*-lactoyl ethanolamine, *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide and *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide are 13, 10, 34 and 31 µg/day, respectively. The estimated daily per capita intakes in the USA and Europe of all flavouring agents in the group are reported in Table 10.

Absorption, distribution, metabolism and elimination

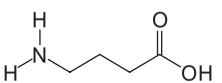
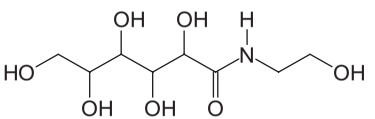
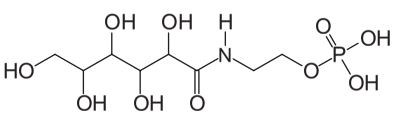
The metabolism of aliphatic and aromatic amines and amides was previously described in the report of the sixty-fifth meeting (Annex 1, reference *178*).

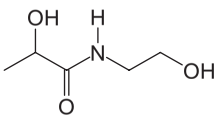
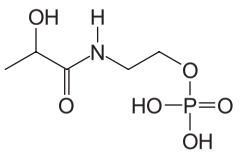
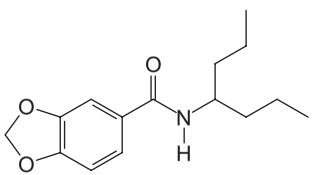
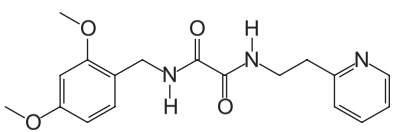
In general, aliphatic and aromatic amines and amides are rapidly absorbed in the gastrointestinal tract and transformed to polar metabolites that are readily eliminated in the urine. Many amines are endogenous and have been identified as normal constituents of urine in humans, including 4-aminobutyric acid (No. 1771).

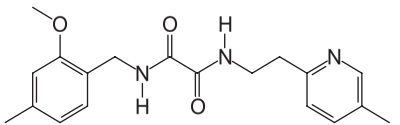
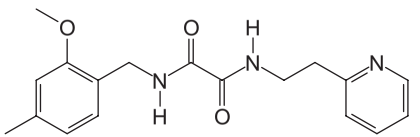
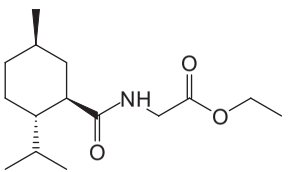
Aliphatic amides have been reported to undergo hydrolysis in mammals; however, the rate of hydrolysis is dependent on the chain length and may involve a number of different enzymes. In relation to the substances in this group of flavouring agents, there is only limited information regarding metabolic pathways for specific substances. The four amides containing ethanolamine (Nos 1772–1775) are hydrolysed mainly in the liver, with only minimal hydrolysis occurring in the stomach or intestines. An *N*-acylethanolamine amidohydrolase has been identified in rat liver that catalyses the hydrolysis of long-chain *N*-acylethanolamines with high efficiency and shorter-chain analogues with lower efficiency. *N*-Gluconyl ethanolamine phosphate (No. 1773) and *N*-lactyl ethanolamine phosphate (No. 1775) are simple phosphate esters that are expected to undergo rapid hydrolysis.

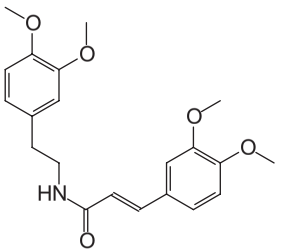
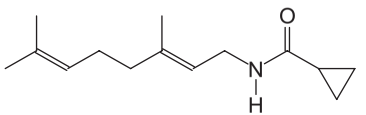
Table 10

Summary of the results of safety evaluations of aliphatic and aromatic amines and amides used as flavouring agents[a,b,c]

Flavouring agent	No.	CAS No. and structure	*Step A3/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 intake
Structural class I						
4-Aminobutyric acid	1771	56-12-2 	No Europe: ND USA: 0.1 Japan: ND	NR	See note 1	No safety concern
N-Gluconyl ethanolamine	1772	686298-93-1 	No Europe: ND USA: 13 Japan: ND	NR	See note 2	No safety concern
N-Gluconyl ethanolamine phosphate	1773	791807-20-0 	No Europe: ND USA: 3 Japan: ND	NR	See note 2	No safety concern

N-Lactoyl ethanolamine	1774	5422-34-4 	No Europe: ND USA: 10 Japan: ND	NR	See note 2	No safety concern
N-Lactoyl ethanolamine phosphate	1775	782498-03-7 	No Europe: ND USA: 5 Japan: ND	NR	See note 2	No safety concern
Structural class III						
N-(Heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide	1767	745047-51-2 	No Europe: 0.01 USA: 0.1 Japan: ND	Yes. The NOEL of 20 mg/kg bw per day is >10 million times the estimated daily intake of *N*-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide when used as a flavouring agent.	See note 2	No safety concern
*N*1-(2,4-Dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide	1768	745047-53-4 	No Europe: 0.01 USA: 0.2 Japan: ND	Yes. The NOEL of 100 mg/kg bw per day is >33 million times the estimated daily intake of *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide when used as a flavouring agent.	See note 2	No safety concern

*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide	1769 745047-94-3 	No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 100 mg/kg bw per day for the related substance *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768) is 500 million times the estimated daily intake of *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide when used as a flavouring agent.	See note 2	No safety concern
*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide	1770 745047-97-6 	No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 100 mg/kg bw per day for the related substance *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768) is 500 million times the estimated daily intake of *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide when used as a flavouring agent.	See note 2	No safety concern
N-[(Ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide	1776 68489-14-5 	No Europe: ND USA: 34 Japan: ND	Yes. The NOEL of 8 mg/kg bw per day for the related substance *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) is >13 000 times the estimated daily intake of *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide when used as a flavouring agent.	See note 3	No safety concern

N-[2-(3,4-Dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide	1777	69444-90-2 	No Europe: ND USA: 0.02 Japan: ND	Yes. The NOEL of 8.36 mg/kg bw per day for the related substance *N*-nonanoyl-4-hydroxy-3-methoxybenzylamide (No. 1599) is >400 000 times the estimated daily intake of *N*-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide when used as a flavouring agent.	See note 2	No safety concern
N-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide	1779	744251-93-2 	No Europe: ND USA: 31 Japan: ND	Yes. The NOEL of 92 mg/kg bw per day is >180 000 times the estimated daily intake of *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide when used as a flavouring agent.	See note 2	No safety concern

CAS, Chemical Abstracts Service; ND, no data reported; NR, not required because consumption of the substance was determined to be of no safety concern at Step A3/B3 of the procedure.

[a] Thirty-six flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *178*).

[b] *Step 1:* Five flavouring agents are in structural class I, and seven flavouring agents are in structural class III.

[c] *Step 2:* Five of the agents in this group (Nos 1771–1775) can be predicted to be metabolized to innocuous products. The remaining 7 agents (Nos 1767–1770, 1776, 1777 and 1779) cannot be predicted to be metabolized to innocuous products.

[d] The thresholds for human intake for structural classes I and III are 1800 and 90 µg/person per day, respectively. All intake values are expressed in µg/day.

Notes to Table 10:

1. Endogenous in mammals and is readily utilized in known metabolic pathways.
2. Amides are subject to limited hydrolysis, with the corresponding ammonium ion or amines entering into known pathways of metabolism and excretion.
3. Anticipated to undergo hydrolysis at the ester moiety followed by conjugate formation and subsequent elimination in the urine.

N-[(Ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide (No. 1776) is hydrolysed in pancreatic juice and rat liver homogenate, but the major route is ester hydrolysis rather than amide hydrolysis. With *N*-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide (No. 1767), there was rapid metabolism in the presence of rat hepatocytes, but no amide hydrolysis products were found. A similar result was found with *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768): rapid metabolism in the presence of rat hepatocytes was observed, and no amide hydrolysis products were found.

No information is available on the metabolism of the other substances in this group of flavouring agents.

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 1771–1775) to structural class I and the remaining seven agents (Nos 1767–1770, 1776, 1777 and 1779) to structural class III.

Step 2. Five flavouring agents in this group (Nos 1771–1775) are predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the A-side of the Procedure. For the remaining substances, there are limited metabolic data available, and they could not be predicted to be metabolized to innocuous products. The evaluation of these seven flavouring agents (Nos 1767–1770, 1776, 1777 and 1779) therefore proceeded via the B-side of the Procedure.

Step A3. The estimated daily per capita exposures for all five 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, these five flavouring agents raise no safety concern when they are used at their currently estimated levels of intake.

Step B3. The estimated daily per capita exposures for the seven flavouring agents in structural class III are below the threshold of concern (i.e. 90 μg/person per day for class III). Accordingly, the evaluation of all seven flavouring agents proceeded to step B4.

Step B4. For *N*-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide (No. 1767), the NOEL of 20 mg/kg bw per day from a 93-day study in rats provides an adequate margin of safety (>10 million) in relation to the currently estimated level of exposure from its use as a flavouring agent in Europe (0.0002 μg/kg bw per day) and in the USA (0.002 μg/kg bw per day).

For *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide (No. 1768), the NOEL of 100 mg/kg bw per day from a 93-day study in rats provides an adequate margin of safety (>33 million) in relation to the currently estimated level of exposure from its use as a flavouring agent in Europe (0.0002 μg/kg bw per day) and in the USA (0.003 μg/kg bw per day). This NOEL is appropriate for the structurally related flavouring agents *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide (No. 1769) and *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl) oxalamide (No. 1770), because they are also oxalamides and are expected to be metabolized by similar pathways. For these structurally related flavouring agents, the NOEL of 100 mg/kg bw per day provides a margin of safety of 500 million in relation to the currently estimated levels of exposure of these flavouring agents in both Europe and the USA (0.0002 μg/kg bw per day).

For *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide (No. 1776), the NOEL of 8 mg/kg bw per day for the structurally related substance *N*-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (No. 1601) from a 28-day study in rats provides an adequate margin of safety (>13 000) in relation to the currently estimated level of exposure from its use as a flavouring agent in the USA (0.6 μg/kg bw per day).

For *N*-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide (No. 1777), the NOEL of 8.36 mg/kg bw per day for the structurally related *N*-nonanoyl-4-hydroxy-3-methoxybenzylamide (No. 1599) from a 90-day study in rats provides an adequate margin of safety (>400 000) in relation to the currently estimated level of exposure from its use as a flavouring agent in the USA (0.02 μg/kg bw per day).

For *N*-3,7-dimethyl-2,6-octadienylcyclopropylcarboxamide (No. 1779), the NOEL of 92 mg/kg bw per day from a 28-day study in rats provides an adequate margin of safety (>180 000) in relation to the currently estimated level of exposure from its use as a flavouring agent in the USA (0.5 μg/kg bw per day).

Table 10 summarizes the evaluations of the 12 aliphatic and aromatic amines and amides in this group.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are predicted to be metabolized by hydrolysis, by oxidative metabolism of amino groups and/or aromatic or heterocyclic rings and/or alkyl side-chains and possibly by conjugation with glucuronic acid. Such metabolic pathways have a high capacity and would not be saturated, even if all flavouring agents were consumed at the same time. A number of the substances in this group that have been evaluated at

this meeting and at the sixty-fifth meeting contain a primary aliphatic amino group (RCH_2NH_2, where R is an alkyl or aryl structure). Such compounds (Nos 1579, 1580, 1582, 1585 and 1588) are predicted to be metabolized by monoamine oxidase. Trimethylamine oxide (No. 1614) is a metabolite of trimethylamine (No. 1610), and *N*-gluconyl ethanolamine (No. 1772) and *N*-lactoyl ethanolamine (No. 1774) are likely metabolites of the corresponding phosphate esters (Nos 1773 and 1775, respectively), with ethanolamine as a possible minor metabolite of all four substances. The combined intakes of the related substances are below the relevant class I threshold. The other substances in this group have diverse structures, with various potential sites of metabolism, and are not likely to be metabolized to common products. The Committee concluded that under the conditions of use as flavouring agents, the combined intake of the substances in this group would not saturate metabolic pathways and the combined intakes would not raise safety concerns.

Consideration of secondary components

Two members of this group of flavouring agents, *N*-lactoyl ethanolamine (No. 1774) and *N*-lactoyl ethanolamine phosphate (No. 1775), have assay values of <95%. Information on the safety of the secondary components of these two 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 of *N*-lactoyl ethanolamine (No. 1774), 2-aminoethanol lactate, is expected to share the same metabolic fate as the main component and is considered not to present a safety concern at the currently estimated level of exposure. The secondary component of *N*-lactoyl ethanolamine phosphate (No. 1775), ammonium formate, is expected to undergo rapid absorption, distribution, metabolism and excretion from the body and is considered not to present a safety concern at the currently estimated level of exposure.

Conclusion

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

The Committee concluded that these 12 flavouring agents, which are additions to the aliphatic and aromatic amines and amides evaluated previously, would not give rise to safety concerns at the currently estimated levels of exposure. The Committee noted, while making this conclusion, that

4-aminobutyric acid (No. 1771) is an endogenous neurotransmitter; however, the level in tissues from flavouring use would be insignificant.

An addendum to the toxicological monograph was prepared.

4.1.8 *Aliphatic alicyclic linear α,β-unsaturated di- and trienals and related alcohols, acids and esters: additional compounds*

The Committee evaluated a group of seven α,β-unsaturated di- and trienals and related alcohols and esters that included four hexadienol derivatives, one heptadienol and two nona- and decatrienal derivatives using the Procedure for the Safety Evaluation of Flavouring Agents (Fig. 1) (Annex 1, reference *131*). None of these substances has previously been evaluated.

The Committee previously evaluated 26 other members of this chemical group of flavouring agents at its sixty-first meeting (Annex 1, reference *166*). The findings from these evaluations were considered in the present evaluation. All 26 substances in that group were concluded to be of no safety concern based on current levels of intake.

Three of the seven flavouring substances (Nos 1784–1786) have been reported to occur naturally in black tea, beans, endive, Lamb's lettuce and fish oil (*27*). Quantitative intake data were not available, and therefore consumption ratios (the ratios of their consumption from natural food sources to their use as flavouring agents) could not be calculated.

Assessment of dietary exposure

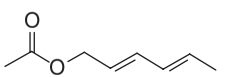
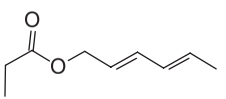
The total annual volume of production of the seven di- and trienals and related alcohols and esters is approximately 3 kg in Europe, 58 kg in the USA and 0.4 kg in Japan (*3*, *4*, *5*). Approximately 88%, 99% and 100% of the total annual volumes in Europe, the USA and Japan, respectively, are accounted for by the four 2,4-hexadienyl esters (Nos 1780–1783) included in this group. The daily per capita intake of each flavouring agent is reported in Table 11. Annual volumes of production of this group of flavouring agents are summarized in Table 12.

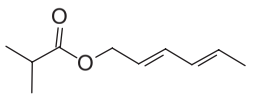
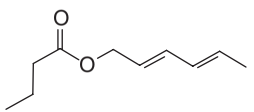
Absorption, distribution, metabolism and elimination

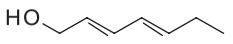
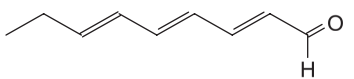
In general, aliphatic esters are rapidly hydrolysed to their component alcohols and carboxylic acids by carboxylesterases. Once hydrolysed, the resulting aliphatic alcohols and carboxylic acids are absorbed in the gastrointestinal tract. The unsaturated alcohols are successively oxidized to the corresponding aldehydes and carboxylic acids, which participate in fundamental biochemical pathways, including the fatty acid pathway and tricarboxylic acid cycle (*51*).

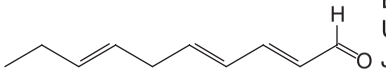
Table 11

Summary of the results of safety evaluations of aliphatic alicyclic linear α,β-unsaturated di- and trienals and related alcohols, acids and esters 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 intake
Structural class I						
2,4-Hexadienyl acetate	1780	1516-17-2 	No Europe: 0.03 USA: 0.01 Japan: 0.03	Yes. The NOEL of 15 mg/kg bw per day (*54*) for the related substance *trans,trans*-2,4-hexadienal (No. 1175) is 30 million times the estimated daily intake of 2,4-hexadienyl acetate when used as a flavouring agent.	See notes 1 and 2	No safety concern
2,4-Hexadienyl propionate	1781	16491-25-1 	No Europe: 0.01 USA: 0.03 Japan: 0.03	Yes. The NOEL of 15 mg/kg bw per day (*54*) for the related substance *trans,trans*-2,4-hexadienal (No. 1175) is 30 million times the estimated	See notes 1 and 2	No safety concern

					daily intake of 2,4-hexadienyl propionate when used as a flavouring agent.		
2,4-Hexadienyl isobutyrate	1782	16491-24-0 	No Europe: 0.3 USA: 7 Japan: 0.03	Yes. The NOEL of 15 mg/kg bw per day (*54*) for the related substance *trans*,*trans*-2,4-hexadienal (No. 1175) is 150 000 times the estimated daily intake of 2,4-hexadienyl isobutyrate when used as a flavouring agent.	See notes 1 and 2	No safety concern	
2,4-Hexadienyl butyrate	1783	16930-93-1 	No Europe: 0.01 USA: 0.1 Japan: 0.03	Yes. The NOEL of 15 mg/kg bw per day (*54*) for the related substance *trans*,*trans*-2,4-hexadienal (No. 1175) is 7.5 million times the estimated daily intake of 2,4-hexadienyl butyrate when used as a flavouring agent.	See notes 1 and 2	No safety concern	

2,4-Heptadien-1-ol	1784	33467-79-7		No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 15 mg/kg bw per day (*54*) for the related substance *trans*,*trans*-2,4-hexadienal (No. 1175) is 75 million times the estimated daily intake of 2,4-heptadien-1-ol when used as a flavouring agent.	See note 2	No safety concern
Nona-2,4,6-trienal	1785	57018-53-8		No Europe: 0.01 USA: ND Japan: ND	Yes. The NOEL of 33 mg/kg bw per day (*55*) for the related substance 2-*trans*-4-*cis*-7-*cis*-tridecadienal (No. 1198) is 165 million times the estimated daily intake of nona-2,4,6-trienal when used as a flavouring agent.	See note 2	No safety concern

2,4,7-Decatrienal	1786	51325-37-2		No Europe: 0.01 USA: 0.01 Japan: ND	Yes. The NOEL of 33 mg/kg bw per day (*55*) for the related substance 2-*trans*-4-*cis*-7-*cis*-tridecadienal (No. 1198) is 165 million times the estimated daily intake of 2,4,7-decatrienal when used as a flavouring agent.	See note 2	No safety concern

CAS, Chemical Abstracts Service; ND, no data.

[a] Twenty-six flavouring agents in this group were previously evaluated by the Committee (Annex 1, reference *166*).

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

[c] *Step 2*: None of the flavouring agents in this group is 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 to Table 11:

1. Hydrolysis to 2,4-hexadienol (No. 1174) and corresponding acids, followed by complete metabolism in the fatty acid pathway or the tricarboxylic acid cycle.
2. 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.

Table 12

Annual volumes of production of aliphatic alicyclic linear α,β-unsaturated di- and trienals and related alcohols, acids and 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
2,4-Hexadienyl acetate (1780)			
Europe	0.3	0.03	0.0005
USA	0.1	0.01	0.0002
Japan	0.1	0.03	0.0004
2,4-Hexadienyl propionate (1781)			
Europe	0.1	0.01	0.0002
USA	0.3	0.03	0.0005
Japan	0.1	0.03	0.0004
2,4-Hexadienyl isobutyrate (1782)			
Europe	3	0.3	0.005
USA	57	7	0.1
Japan	0.1	0.03	0.0004
2,4-Hexadienyl butyrate (1783)			
Europe	0.1	0.01	0.0002
USA	1	0.1	0.002
Japan	0.1	0.03	0.0004
2,4-Heptadien-1-ol (1784)			
Europe	0.1	0.01	0.0002
USA	0.1	0.01	0.0002
Japan	ND	NA	NA
Nona-2,4,6-trienal (1785)			
Europe	0.1	0.01	0.0002
USA	ND	NA	NA
Japan	ND	NA	NA
2,4,7-Decatrienal (1786)			
Europe	0.1	0.01	0.0002
USA	0.1	0.01	0.0002
Japan	ND	NA	NA
Total			
Europe	3		
USA	58		
Japan	0.4		

NA, not applicable; ND, no data reported.

[a] From references *3*, *4*, *5*. Total poundage values of <1 kg reported in the surveys (*3*, *4*, *5*) 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 correction factor = 0.6 for NAS surveys in the USA and 0.8 for the surveys by FEMA USA, Europe and Japan, representing the assumption that only 60% or 80% of the annual flavour volume was reported in the poundage surveys, respectively.
Intake (μg/kg bw per day) calculated as follows:
(μg/person per day)/body weight, where body weight = 60 kg. Slight variations may occur as a result of rounding.

It is anticipated that dienals and trienals will be biotransformed in humans by oxidation to the corresponding acids, which may undergo β-oxidative cleavage and complete metabolism via the tricarboxylic acid cycle. An alternative minor pathway may involve conjugation of the unsaturated aldehyde or corresponding acid with glutathione, followed by excretion as the mercapturic acid derivative.

α,β-Unsaturated aldehydes are formed endogenously by lipid peroxidation of polyunsaturated fatty acids (*52*) or can be consumed as naturally occurring constituents of food (*27*) and, to a minor extent, as added flavouring agents. Under conditions of glutathione depletion and oxidative stress, high cellular concentrations of α,β-unsaturated aldehydes have been shown to form adducts with bases in DNA, cause cytohistopathology and induce DNA fragmentation during apoptosis. However, as considered at the evaluation of the first 26 members of this chemical group (Annex 1, reference *166*), metabolic evidence indicates that low levels of intake of α,β-unsaturated aldehydes are metabolized in the high-capacity β-oxidation pathway or via glutathione conjugation.

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 substances, the Committee assigned all seven flavouring agents (Nos 1780–1786) to structural class I (*38*).

Step 2. None of the flavouring agents in this group is expected to be metabolized to innocuous products. The evaluation of all flavouring agents in this group therefore proceeded via the B-side of the Procedure.

Step B3. The estimated daily per capita intakes of the seven flavouring agents in this group, of which all are in structural class I, are below the threshold of concern (i.e. 1800 μg/person per day for class I).

Step B4. The NOEL for the structurally related substance *trans*,*trans*-2,4-hexadienal (No. 1175) is appropriate to evaluate 2,4-hexadienyl acetate (No. 1780), 2,4-hexadienyl propionate (No. 1781), 2,4-hexadienyl isobutyrate (No. 1782), 2,4-hexadienyl butyrate (No. 1783) and 2,4-heptadien-1-ol (No. 1784), since these hexa- and heptadienol derivatives are structurally related and expected to undergo similar pathways of metabolism. The NOEL of 15 mg/kg bw per day for the related substance *trans*,*trans*-2,4-hexadienal (No. 1175) in rats from a 14-week study (*53*) provides an adequate margin of safety of 30 million times the currently estimated intakes of 2,4-hexadienyl acetate and 2,4-hexadienyl propionate from their uses as flavouring substances. For 2,4-hexadienyl isobutyrate, the NOEL provides an adequate margin of safety of 150 000 in relation to currently estimated intakes of this substance from its use as a flavouring agent. The NOEL provides adequate margins of safety of 7.5 million and 75 million in relation to the currently estimated intakes of 2,4-hexadienyl butyrate and 2,4-heptadien-1-ol, respectively, from their uses as flavouring substances.

The NOEL for the structurally related substance 2-*trans*-4-*cis*-7-*cis*-trideca-trienal (No. 1198) is appropriate to evaluate nona-2,4,6-trienal (No. 1785) and 2,4,7-decatrienal (No. 1786), because the substances are structurally related and expected to undergo similar pathways of metabolism. The NOEL of 33 mg/kg bw per day for the related substance 2-*trans*-4-*cis*-7-*cis*-tridecatrienal in rats from a 4-week study (*54*) provides adequate margins of safety of 165 million in relation to the currently estimated intakes of both nona-2,4,6-trienal and 2,4,7-decatrienal from their uses as flavouring substances.

Table 11 summarizes the evaluations of the seven α,β-unsaturated di- and trienals and related alcohols and esters in this group.

Consideration of combined intakes from use as flavouring agents

The flavouring agents in this group are predicted to be metabolized by hydrolysis and/or oxidative metabolism of alcohol and aldehyde groups and alkyl side-chains and by conjugation with glutathione. These pathways have a high capacity and would not be saturated, even if all flavouring agents were consumed at the same time. A number of the substances in this group that have been evaluated at this meeting and at the sixty-first meeting are predicted to be metabolized to common metabolites. Common metabolites (and their precursors) are sorbic acid (Nos 1174, 1175, 1177, 1178 and 1780–1783), 2,4-nonenoic acid (Nos 1183 and 1185), *cis*- or *trans*-2,6-nonenoic acid (Nos 1184 and 1186–1188), *cis*- or *trans*-2,4-decadienoic acid (Nos 1189–1192 and 1194) and 2,4,7-decatrienoic acid (Nos 1193 and 1786). The alcohols produced by hydrolysis would be oxidized to the corresponding aldehydes

and subsequently to the above acids. The combined intakes of substances with a common metabolite were below the threshold for class I. The Committee concluded that under the conditions of use as flavouring agents, the combined intake 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

All substances in this group have minimum assay values of 95% or greater.

Conclusion

In the previous evaluation of substances in this group, studies of acute toxicity, short-term toxicity (14 days to 13 weeks), long-term toxicity and carcinogenicity, genotoxicity and reproductive toxicity were available. None raised safety concerns. The results of a new study on acute toxicity were consistent with findings previously evaluated.

The Committee concluded that these seven flavouring substances, which are additions to the group of α,β-unsaturated di- and trienals and related alcohols evaluated previously, would not give rise to safety concerns at the currently estimated levels of intake.

No addendum to the toxicological monograph was prepared.

4.2 Specifications of purity for flavouring agents

4.2.1 *Specifications for flavouring agents evaluated for the first time*

The Committee received information for specifications for 168 flavouring agents. Two of these flavours were duplicated, and the Committee questioned whether six substances in Group 4 (Nos 1720–1725; see section 4.1.4) have flavouring properties. As a result, specifications were not prepared for these eight materials. Full specifications were prepared for a total of 160 flavouring agents.

4.2.2 *Revision of existing specifications for flavouring agents*

4.2.2.1 *Maltol and ethyl maltol*

These substances have flavouring agent specifications under Nos 1480 and 1481 and tentative specifications in the traditional food additives format. These latter specifications were revised at the present meeting and the tentative designations removed. The Committee also revised the flavouring agent specifications. For more details, see section 3.2.1.

4.2.2.2 *Maltyl isobutyrate, 3-acetyl-2,5-dimethylfuran and 2,4,5-trimethyl-delta-oxazoline (Nos 1482, 1506 and 1559)*

The Committee reviewed the specifications for these three substances, all of which were designated as tentative at its sixty-fifth meeting (Annex 1, reference *178*) pending further information to enable the ranges for specific gravity and, in the case of No. 1559, refractive index to be reduced.

New information was provided on the ranges for specific gravity and refractive index for all three substances, as a result of which the three specifications were revised and their tentative designations removed.

4.2.2.3 *Method of assay for the sodium salts of certain flavouring agents*

The specifications for four flavouring agents were designated tentative at the sixty-fifth meeting (Annex 1, reference *178*), pending receipt of information on the method of assay by high-performance liquid chromatography (HPLC). A suitable method was received, and the specifications were revised. The four flavourings are:

No.	Name
631.2	Sodium 3-methyl-2-oxobutanoate
632.2	Sodium 3-methyl-2-oxopentanoate
633.2	Sodium 4-methyl-2-oxopentanoate
1479	Sodium 2-oxo-3-phenylpropionate

The tentative designations were removed from these specifications. It was noted that the method also applies to the acid forms of these salts (Nos 631, 632, 633 and 1478).

At the present meeting, the Committee renumbered 631.2, 632.2 and 633.2 as 631.1, 632.1 and 633.1, respectively.

Additional information related to the specifications for the flavouring agents with Nos 631, 632 and 633 was also provided to the Committee, and these specifications were revised.

4.2.2.4 *Monomenthyl glutarate (No. 1414)*

The Committee was informed that because of new preparation procedures, the material now being produced may not meet the present specifications. Whereas the present specification has 72% (minimum) monomenthyl glutarate, the chemical assays now reported indicate that the monomenthyl glutarate varies from 58% to 80% and dimenthyl glutarate varies from 8% to 41%. The Committee concluded that such a mixture requires different specifications from those published for flavouring agent No. 1414, but more information is required before new specifications can be prepared. Meanwhile, it was agreed to maintain the existing specifications.

5. Contaminants

5.1 Aflatoxins: impact of different hypothetical limits for almonds, Brazil nuts, hazelnuts, pistachios and dried figs

5.1.1 *Explanation*

The aflatoxins (AFL) were evaluated by the Committee at its thirty-first, forty-sixth, forty-ninth and fifty-sixth meetings (Annex 1, references *77*, *122*, *131* and *152*). At the thirty-first meeting, the Committee considered AFL to be a potential human carcinogen and urged that dietary exposure to AFL be reduced to the lowest practicable levels, so as to reduce the potential risk as far as possible. IARC also concluded that naturally occurring AFL are carcinogenic to humans. At its forty-sixth meeting, the Committee considered estimates of the carcinogenic potency of AFL and the potential risk associated with their intake. In view of the value of such estimates, the Committee recommended that this task be continued at a subsequent meeting. At its forty-ninth meeting, the Committee analysed the effects of applying hypothetical standards for contamination in maize and groundnuts with AFL B_1 (AFB_1; 10 and 20 µg/kg) and concluded that reducing the standard from 20 to 10 µg/kg would not result in any observable difference in the rates of liver cancer. At its fifty-sixth meeting, the Committee concluded that the potency of AFL in hepatitis B virus surface antigen positive ($HBsAg^+$) individuals is substantially higher than the potency in hepatitis B virus surface antigen negative ($HBsAg^-$) individuals and that the liver cancer burden could best be reduced by giving priority to vaccination campaigns against hepatitis B and to prevention of infection with hepatitis C; the latter would require greater control of blood and blood products.

CCFAC at its thirty-eighth session (*7*) requested that the Committee conduct a dietary exposure assessment for total aflatoxins (AFT) from consumption of tree nuts (ready-to-eat)—in particular, almonds, Brazil nuts, hazelnuts and pistachios—and analyse the impact on dietary exposure of hypothetical MLs of 4, 8, 10 and 15 µg/kg with consideration of the overall AFT dietary exposure, including consumption of maize and groundnuts. An additional request

was received by the Committee to take into account in its assessment an additional hypothetical ML of 20 μg/kg.

In this evaluation, the sum of AFL B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2) is referred to as AFT. The Committee agreed that this assessment applies to the edible parts of almonds (Codex food and feed classification number TN 0660) of cultivars grown from *Prunus amygdalus*, to Brazil nuts (TN 0662) ("white almonds") of the species *Bertholletia excelsa*, to the "common edible hazelnuts" (TN 0666) from *Corylus avellana* intended for direct consumption and to pistachio nuts (TN 0675) of cultivars grown from *Pistacia vera*. Additionally, the evaluation considered dried figs (DF 0297) from ripe fruits of cultivars grown from *Ficus carica* and intended for direct consumption. It does not apply to dried figs intended for processing.

AFL occurrence and concentration data, submitted from 22 EU Member States for the European Food Safety Authority (EFSA) risk assessment requested by the European Commission in 2006, were available for this evaluation. Australia, Brazil, the Islamic Republic of Iran, Japan, Turkey, United Arab Emirates and the USA also submitted data on AFL contamination. In total, the Committee had access to over 100 000 data points for its analyses. Other data on contamination by these toxins have been taken from published literature, but they were not used to calculate dietary exposure because the disaggregated data were not available. Rather, they were used to reinforce the analysis made in the document.

The results of studies relevant to a toxicological evaluation, particularly metabolic and epidemiological studies, published since the last JECFA risk assessment of AFL, did not alter that assessment and indeed lent support to the conclusions reached in that assessment. They were not further considered in this current assessment.

5.1.2 *Analytical methods*

In the studies evaluated by the Committee at its present meeting, it was usually clear which AFT analytical method had been used. However, in the submitted data, detection and quantification limits for AFT were calculated in different ways. One method defined the LOD of AFT as twice the value of the LOD of AFB_1, whereas the second used the sum of the LODs of AFB_1, AFB_2, AFG_1 and AFG_2. The Committee concluded that both definitions overestimate the LOD of AFT, resulting in conservative estimates of the exposure to AFT for the upper-bound estimate. The Committee also concluded that it was better to restrict data used in the dietary exposure assessment to those with validated recoveries greater than 70% than to correct for lower recoveries. The Committee also noted that surveillance data should be accompanied by

a clear description of the analytical method used; recoveries of the analytical methodology chosen should be specific to the food matrix tested; and LODs and limits of quantification (LOQs) should be provided with the definitions used to derive them. Efforts should be made to harmonize the nomenclature and the methodologies by which the LOD and LOQ were calculated.

The combination of liquid chromatography with mass spectrometry is one useful technique for the confirmation of the presence of AFL in foodstuffs. Although some methods are already implemented in routine analysis, the limited number of reference materials, high investment costs and lack of the required sensitivity could be a barrier to use for AFL surveillance, because it was noted that for accurate dietary exposure assessments, the LOD/LOQ should be as low as technically possible. This is due to the fact that many foods that might be expected to contain AFL do not contain *detectable* AFL contamination, and the default value assigned to those censored samples will affect the estimated dietary exposures (upper-bound estimates only).

5.1.3 *Sampling protocols*

Almost all of the submitted data on AFT were derived using sampling plans designed for regulatory purposes. The producing countries that submitted data to the Committee presented sampling plans similar to or the same as those following EC No. 401/2006 for the determination of AFL, which includes edible nuts and dried figs. It was observed that in some producing countries, there are two sampling plans: one for commodity to be exported to the EU, and the second for commodity to be exported to other countries with less strict regulations. There remains a need for harmonized sampling plans, both between different countries and within the same country. The Committee noted that AFL sampling plans should be determined by data relating to contamination distributions and uncertainties within the particular foodstuff. The resulting knowledge of the uncertainty among sample test results should allow each country to refine its sampling plans using, for example, larger sample sizes and/or fewer analytical repetitions in order to meet harmonized criteria. The Committee noted that the data received for this analysis were robust.

5.1.4 *Effects of processing*

Although AFL are highly stable, studies have indicated that they are degraded in contaminated food by heat treatment. For example, the roasting of pistachio nuts at 150 ºC for 30 min reduced AFL levels by 63% when the initial level was 44 μg AFB_1/kg, 24% when the initial level was 213 μg AFB_1/kg, 17% when the initial level was 21.9 μg AFB_1/kg and 47% when the initial level was 18.5 μg AFB_2/kg.

5.1.5 *AFL occurrence and levels in food commodities and the potential effect of MLs in almonds, Brazil nuts, hazelnuts, pistachios and dried figs*

AFL occurrence data on almonds, Brazil nuts, hazelnuts, pistachios and dried figs were obtained from both producing and importing countries. The Committee decided to base the assessment of the impact of different MLs for AFT for almonds, Brazil nuts, hazelnuts, pistachios and dried figs (4, 8, 10, 15 and 20 µg/kg) on data provided by producing countries, as these are more likely to represent the actual occurrence of AFL in the commodities. The primary producing countries[9] were, for almonds, the USA (42% of the world market); for Brazil nuts, Latin America (100%); for hazelnuts, Turkey (70%); for pistachios, the Islamic Republic of Iran (65%); and for dried figs, Turkey (63% for dried fruits). Turkey is the primary producing country for hazelnuts, but the Committee received no data on AFT levels in hazelnuts from Turkey; therefore, the Committee chose to use all of the submitted data supplied by the EU, the USA and Japan for its analyses.

The mean concentrations of AFT in nuts and dried figs in the main producing countries were, for almonds, 2 µg/kg; for Brazil nuts, 20 µg/kg; for hazelnuts, 2 µg/kg; for pistachios, 54 µg/kg; and for dried figs, 1 µg/kg. The effects of the theoretical full enforcement of MLs (all samples above the ML would be excluded from the distribution) at 20, 15, 10, 8 and 4 µg/kg are shown in Table 13. The reductions in mean AFT concentrations would be approximately 2- to 3-fold for almonds, 10-fold for Brazil nuts, 2- to 4-fold for hazelnuts, 10- to 50-fold for pistachios and 2-fold for dried figs. The corresponding proportion of rejected samples would be 1–3% for almonds, 11–17% for Brazil nuts, 1–7% for hazelnuts, 40–60% for pistachios and 1–3% for dried figs.

Table 13
Impact of different hypothetical ML scenarios for AFT on the mean AFT level and the corresponding proportion of rejected samples from the producing countries on the world market for tree nuts and dried figs

Scenario	Mean AFT level, µg/kg (proportion of rejected samples, %)					
	No MLs	ML 20 µg/kg	ML 15 µg/kg	ML 10 µg/kg	ML 8 µg/kg	ML 4 µg/kg
Almonds	2.0 (0)	0.8 (1)	0.7 (2)	0.7 (2)	0.6 (3)	0.6 (3)
Brazil nuts	20 (0)	2.4 (11)	2.1 (13)	1.9 (15)	1.8 (16)	1.7 (17)
Hazelnuts	1.9 (0)	1.0 (1)	0.9 (2)	0.8 (3)	0.7 (4)	0.6 (7)
Pistachios	54 (0)	4.4 (40)	3.4 (44)	2.4 (49)	2.0 (53)	1.2 (61)
Dried figs	1.0 (0)	0.6 (1)	0.6 (1)	0.5 (1)	0.5 (2)	0.4 (3)

[9] FAOSTAT 2007 (http://faostat.fao.org/).

5.1.6 *Assessment of dietary exposure*

At the regional level, published studies reported that estimated mean dietary exposures to AFT for the general population from all food sources were 0.93−2.4 ng/kg bw per day in Europe, 3.5−180 ng/kg bw per day in Africa, 0.3−53 ng/kg bw per day in Asia and 2.7 ng/kg bw per day in the USA.

In this assessment, mean lower- and upper-bound scenarios have been used in making the dietary exposure estimates employing the 13 GEMS/Food Consumption Cluster Diets (Tables 13, 14, 15 and 16). The lower bound was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as appropriate.

The Committee employed the 13 GEMS/Food Consumption Cluster Diets to make international estimates of dietary AFT exposure from all sources. These were estimated to range from 0.4−0.7 ng/kg bw per day (cluster K) to 3.0−3.7 ng/kg bw per day (cluster J), by assuming a body weight of 60 kg and using the lower-bound/upper-bound approach. The mean total dietary exposure to AFT from maize, groundnuts, oilseeds and cocoa products made the greatest contribution to total exposure in all cluster diets (Table 15). Dietary AFB_1 exposure ranged from 0.3−0.5 ng/kg bw per day to 2.3−2.8 ng/kg bw per day for the same clusters (Table 14).

Almonds, Brazil nuts, hazelnuts, pistachios and dried figs

The mean contribution to dietary AFT exposure from consumption of almonds, Brazil nuts, hazelnuts, pistachios and dried figs ranged from 0 ng/kg bw per day (clusters A, G, I and J; nut consumption reported as zero for these clusters) up to 0.8 ng/kg bw per day (clusters B and D). In five cluster diets (B, C, D, E and M), the contribution from almonds, Brazil nuts, hazelnuts and pistachios was higher than 5% of the overall dietary exposure to AFT (Table 14).

Pistachios were the main contributor to dietary AFT exposure from tree nuts in all five cluster diets, ranging from 0.2 to 0.8 ng/kg bw per day, equivalent to 7−45% of the total AFT from all sources (Table 16). Almonds, Brazil nuts and hazelnuts contributed up to 0.1 ng/kg bw per day, and dried figs less than 0.01 ng/kg bw per day, in all Consumption Cluster Diets.

Foods other than tree nuts and dried figs

In order to evaluate the relative contribution of tree nuts and dried figs to the overall AFT exposure, the Committee considered other foods known to contribute to the overall exposure to AFT in humans. Occurrence data and dietary exposures to AFT from these other foods were described. Food commodities included in the mean overall exposure were maize, groundnuts (i.e. peanuts)

Table 14

Mean estimates of dietary exposure to AFB_1 and AFT from all food sources for the 13 GEMS/Food Consumption Cluster Diets taking into consideration hypothetical ML scenarios for AFT (no MLs; 4, 8, 10, 15 and 20 µg/kg) in tree nuts and the contribution of tree nuts to total AFT dietary exposure

Scenario[a]		Mean dietary exposure (ng/kg bw per day) for the 13 GEMS/Food Consumption Cluster Diets												
		A	B	C	D	E	F	G	H	I	J	K	L	M
No ML	AFB_1	0.9–1.2	1.7–2.3	1.1–1.7	1.2–1.4	1.3–1.7	0.6–0.8	1.0–1.1	1.0–1.9	1.1–1.8	2.3–2.8	0.3–0.5	0.6–0.9	1.3–1.8
	AFT	1.1–1.7	2.1–3.2	1.5–2.5	1.4–1.8	1.7–2.3	0.7–1.1	1.3–1.6	1.4–2.8	1.4–2.7	3.0–3.7	0.4–0.7	0.8–1.3	1.7–2.5
	All tree nuts (% AFT)	0.0	24.6	20.0	45.0	16.8	3.7	0.0	3.3	0.0	0.0	4.3	0.8	9.3
ML 20 µg/kg	AFB_1	0.9–1.2	1.1–1.7	0.8–1.3	0.5–0.7	1.0–1.4	0.6–0.8	1.0–1.1	1.0–1.8	1.1–1.8	2.3–2.8	0.3–0.5	0.6–0.9	1.2–1.6
	AFT	1.1–1.7	1.5–2.5	1.1–2.0	0.7–1.1	1.3–2.0	0.7–1.1	1.3–1.6	1.3–2.7	1.4–2.7	3.0–3.7	0.4–0.7	0.8–1.3	1.5–2.3
	All tree nuts (% AFT)	0.0	4.7	2.6	6.3	3.2	1.6	0.0	0.3	0.0	0.0	0.6	0.3	1.1
ML 15 µg/kg	AFB_1	0.9–1.2	1.1–1.7	0.7–1.3	0.5–0.7	1.0–1.4	0.6–0.8	1.0–1.1	1.0–1.8	1.1–1.8	2.3–2.8	0.3–0.5	0.6–0.9	1.2–1.6
	AFT	1.1–1.7	1.4-2.5	1.1–2.0	0.6–1.1	1.3–2.0	0.7–1.1	1.3–1.6	1.3–2.7	1.4–2.7	3.0–3.7	0.4–0.7	0.8–1.3	1.5–2.3
	All tree nuts (% AFT)	0.0	4.1	2.2	5.0	2.8	1.5	0.0	0.3	0.0	0.0	0.5	0.3	0.9
ML 10 µg/kg	AFB_1	0.9–1.2	1.1–1.7	0.7–1.3	0.5–0.7	1.0–1.4	0.6–0.8	1.0–1.1	1.0–1.8	1.1–1.8	2.3–2.8	0.3–0.5	0.6–0.9	1.2–1.6
	AFT	1.1–1.7	1.4–2.5	1.0–2.0	0.6–1.1	1.3–2.0	0.7–1.1	1.3–1.6	1.3–2.7	1.4–2.7	3.0–3.7	0.4–0.7	0.8–1.3	1.5–2.3
	All tree nuts (% AFT)	0.0	3.3	1.7	3.6	2.3	1.4	0.0	0.2	0.0	0.0	0.4	0.2	0.7
ML 8 µg/kg	AFB_1	0.9–1.2	1.1–1.7	0.7–1.3	0.5–0.7	1.0–1.4	0.6–0.8	1.0–1.1	1.0–1.8	1.1–1.8	2.3–2.8	0.3–0.5	0.6–0.9	1.2–1.6
	AFT	1.1–1.7	1.4–2.5	1.1–2.0	0.6–1.1	1.3–2.0	0.7–1.1	1.3–1.6	1.3–2.7	1.4–2.7	3.0–3.7	0.4–0.7	0.8–1.3	1.5–2.3
	All tree nuts (% AFT)	0.0	2.9	1.5	3.0	2.1	1.2	0.0	0.2	0.0	0.0	0.4	0.2	0.7
ML 4 µg/kg	AFB_1	0.9–1.2	1.1–1.7	0.7–1.3	0.4–0.7	1.0–1.4	0.6–0.8	1.0–1.1	1.0–1.8	1.1–1.8	2.3–2.8	0.3–0.5	0.6–0.9	1.2–1.6
	AFT	1.1–1.7	1.4–2.5	1.1–2.0	0.6–1.1	1.3–2.0	0.7–1.1	1.3–1.6	1.3–2.7	1.4–2.7	3.0–3.7	0.4–0.7	0.8–1.3	1.5–2.3
	All tree nuts (% AFT)	0.0	2.3	1.1	1.9	1.7	1.1	0.0	0.1	0.0	0.0	0.4	0.2	0.5

[a] Lower- and upper-bound scenarios have been used in making the dietary exposure estimates for overall exposure and all tree nuts. The lower bound was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as appropriate. “All tree nuts” includes dried figs, which contributed less than 0.3% of the AFT dietary exposure in all scenarios. % AFT is the contribution from almonds, Brazil nuts, hazelnuts, pistachios and dried figs to total AFT dietary exposure (upper-bound scenario only).

Table 15

Summary of the mean overall estimates of international dietary exposure to AFT from other contributing food sources (lower- and upper-bound scenarios) for the 13 GEMS/Food Consumption Cluster Diets and the corresponding exposure from each food commodity

	Dietary exposure to AFT (ng/kg bw per day) for the 13 GEMS/Food Consumption Cluster Diets												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Overall exposure from other sources	1.1–1.7	1.3–2.4	1.0–2.0	0.6–1.0	1.3–1.9	0.7–1.1	1.3–1.6	1.3–2.7	1.4–2.7	3.0–3.7	0.4–0.7	0.7–1.3	1.5–2.2
Mean dietary exposure to AFT from individual food sources[a]													
Maize	0.2–0.7	0.4–1.0	0.3–0.9	0.1–0.2	0.1–0.3	0.04–0.10	0.1–0.2	0.8–2.1	0.6–1.7	0.2–0.4	0.2–0.5	0.2–0.4	0.3–0.7
Groundnuts	0.7–0.7	0.4–0.4	0.3–0.3	0.1–0.1	0.5–0.5	0.2–0.2	0.9–1.0	0.3–0.3	0.6–0.6	2.6–2.9	0.1–0.1	0.1–0.1	0.8–0.9
Oilseeds	0.1–0.2	0.4–0.6	0.2–0.3	0.3–0.5	0.4–0.6	0.2–0.3	0.1–0.2	0.1–0.2	0.1–0.2	0.1–0.1	0.02–0.04	0.4–0.6	0.1–0.2
Cocoa products	0.02–0.04	0.1–0.2	0.03–0.1	0.04–0.1	0.2–0.4	0.2–0.4	0.02–0.04	0.1–0.1	0.03–0.04	0.02–0.03	0.1–0.1	0.1–0.1	0.2–0.3
Other nuts	0.0–0.0	0.04–0.1	0.0–0.0	0.0–0.01	0.01–0.02	0.0–0.0	0.01–0.02	0.01–0.01	0.0–0.0	0.0–0.0	0.0–0.0	0.02–0.03	0.01–0.02
Dried fruits other than figs	0.0–0.01	0.02–0.1	0.1–0.3	0.02–0.1	0.01–0.03	0.01–0.03	0.0–0.01	0.0–0.0	0.0–0.0	0.01–0.04	0.0–0.0	0.0–0.01	0.01–0.04
Butter of Karité nut	0.01–0.02	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.02	0.0–0.0	0.0–0.0	0.0–0.0
Peanut oil	0.02–0.03	0.01–0.01	0.01–0.01	0.0–0.0	0.02–0.02	0.01–0.01	0.04–0.05	0.0–0.0	0.02–0.02	0.1–0.1	0.0–0.0	0.0–0.0	0.01–0.01
Spices	0.07–0.08	0.03–0.03	0.07–0.07	0.02–0.03	0.1–0.1	0.03–0.03	0.1–0.1	0.1–0.1	0.04–0.04	0.04–0.04	0.01–0.01	0.02–0.02	0.1–0.1

[a] Lower- and upper-bound scenarios have been used in making the dietary exposure estimates for overall exposure and individual food sources. The lower bound was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as appropriate.

Table 16

Mean estimates of dietary exposure to AFT from almonds, Brazil nuts, hazelnuts and pistachios for the 13 GEMS/Food Consumption Cluster Diets, taking into consideration the impact of different hypothetical ML scenarios for AFT (no MLs; 4 and 20 µg/kg) in tree nuts

		Dietary exposure to AFT (ng/kg bw per day) for the 13 GEMS/Food Consumption Cluster Diets												
		A	B	C	D	E	F	G	H	I	J	K	L	M
Overall exposure from other sources[a]		1.1–1.7	1.3–2.4	1.0–2.0	0.6–1.0	1.3–1.9	0.7–1.1	1.3–1.6	1.3–2.7	1.4–2.7	3.0–3.7	0.4–0.7	0.7–1.3	1.5–2.2
Scenario[b]														
No ML	All tree nuts	0.0–0.0	0.8–0.8	0.5–0.5	0.8–0.8	0.4–0.4	0.04–0.04	0.0–0.0	0.1–0.1	0.0–0.0	0.0–0.0	0.03–0.03	0.01–0.01	0.2–0.2
	Pistachios (% AFT)	0.0	20.1	18.4	44.8	12.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	7.4
	Other tree nuts (% AFT)	0.0	4.4	1.7	0.2	4.9	3.7	0.0	0.1	0.0	0.0	4.3	0.8	1.9
ML 20 µg/kg	All tree nuts	0.0–0.0	0.1–0.1	0.1–0.1	0.1–0.1	0.1–0.1	0.02–0.02	0.0–0.0	0.01–0.01	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.03–0.03
	Pistachios (% AFT)	0.0	2.0	1.8	6.1	1.1	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.6
	Other tree nuts (% AFT)	0.0	2.7	0.8	0.2	2.1	1.6	0.0	0.0	0.0	0.0	0.6	0.3	0.5
ML 4 µg/kg	All tree nuts	0.0–0.0	0.1–0.1	0.02–0.02	0.02–0.02	0.03–0.03	0.01–0.01	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.0–0.0	0.01–0.01
	Pistachios (% AFT)	0.0	0.6	0.5	1.8	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2
	Other tree nuts (% AFT)	0.0	1.7	0.6	0.1	1.4	1.1	0.0	0.0	0.0	0.0	0.4	0.2	0.3

[a] Mean concentration as reported for other contributing food sources to the overall dietary exposure to AFT and the corresponding food consumption from the 13 GEMS/Food Consumption Cluster Diets.

[b] Lower- and upper-bound scenarios have been used in making the dietary exposure estimates for overall exposure and all tree nuts. The lower bound was calculated using 0 for non-detects or the LOD for trace values, whereas the upper bound was calculated using either the LOD or LOQ, as appropriate. “All tree nuts” includes dried figs, which contributed less than 0.3% of the AFT dietary exposure in all scenarios. % AFT is the contribution from almonds, Brazil nuts, hazelnuts, pistachios and dried figs to the total AFT dietary exposure (upper-bound scenario only).

and other nuts (i.e. walnuts, cashews, chestnuts, macadamia nuts, pecans), dried fruits other than figs (apricots, plums, grapes, dates and others), spices, cocoa and cocoa products (cocoa mass, cocoa butter, cocoa powder), peanut butter, peanut cream, oilseeds and butter of Karité nut.

The majority of the data included in the estimation of dietary AFT exposure from other food sources came from the EU. The Committee noted that the European data do not reflect the actual mean values in other world regions for some foods considered here, as the mean concentration of AFT in the EU takes into account fewer highly contaminated samples due to existing EU MLs compared with regions with higher MLs or lack of enforcement.

The mean concentrations of AFB_1 and AFT were less than 1 µg/kg for most foods, except spices, cocoa products, groundnuts and butter of Karité nut, where mean levels ranged between 2 and 4 µg/kg.

The Committee noted that different concentrations in rice were reported in different regions (producing and non-producing countries), with mean AFT levels around 0.6−1.0 µg/kg in the EU, 0.2−1.2 µg/kg in the Republic of Korea and 0.1−0.2 µg/kg in Qatar, with no reports of detected levels in other regions, including Japan and Argentina. High AFT levels, such as those for peanuts or maize, have never been reported in rice; the highest reliably reported levels are less than 10 µg/kg. Because of uncertainties in the data, rice was not included in estimating overall dietary exposures to AFT for comparison with the contribution from almonds, Brazil nuts, hazelnuts, pistachios and dried figs. In regions where rice is a major component of the diet, any low levels of AFT in rice may lead to its being a major contributor to total dietary exposure to AFT, even though that exposure may be low when compared with that in other regions.

5.1.7 *Effect of hypothetical MLs in almonds, Brazil nuts, hazelnuts, pistachios and dried figs on dietary exposure*

The Committee evaluated the impact on dietary exposure to AFT of setting hypothetical MLs of 4, 8, 10, 15 or 20 µg/kg for AFT in almonds, Brazil nuts, hazelnuts, pistachios and dried figs. For dried figs and tree nuts other than pistachios, the contribution to total AFT dietary exposure is less than 5%, regardless of whether an ML is in place or not. This is explained by the fact that the main part of the dietary exposure to AFT comes from other food sources (Tables 14, 15 and 16).

Using the five cluster diets where almonds, Brazil nuts, hazelnuts, pistachios and dried figs contribute more than 5% to dietary AFT exposure (clusters B, C, D, E and M), and assuming a body weight of 60 kg, the Committee estimated that an enforced ML of 20, 15, 10, 8 or 4 µg/kg results in dietary

exposures to AFT ranging from 0.12, 0.10, 0.08, 0.07 and 0.06 ng/kg bw per day in the cluster with the highest exposure (D) to 0.03, 0.02, 0.02, 0.02 and 0.01 ng/kg bw per day in the cluster with the lowest exposure (M).

United Kingdom food consumption data for vegetarians and vegans showed that for high-level consumers of almonds, Brazil nuts, hazelnuts and pistachios, enforcing an ML of 20 µg/kg reduces total AFT dietary exposure when compared with no ML. Setting a lower ML would have little impact compared with the ML of 20 µg/kg. The dietary exposure from tree nuts assuming no ML was estimated to be 5.8 ng/kg bw per day. The estimate with an ML of 20 µg/kg would be 0.5 ng/kg bw per day, and with an ML of 4 µg/kg would be 0.2 ng/kg bw per day.

In these analyses, the contribution from tree nuts to the total AFT dietary exposures in all five cluster diets, whatever the ML scenario (4, 8, 10, 15 or 20 µg/kg), will remain below 0.1 ng/kg bw per day, compared with <0.8 ng/kg bw per day for the scenario with no MLs. The highest decrease in AFT exposure results from the contribution from pistachios to total AFT dietary exposure when setting an ML at 20 µg/kg in comparison with no ML.

The Committee also noted that in all these different ML scenarios, dried figs were included (dietary exposures not shown in tables). However, the contribution of dried figs (<0.01 ng/kg bw per day) to total AFT dietary exposure estimates in all Consumption Cluster Diets, whatever the ML scenario, would be less than 0.3% of the overall dietary AFT exposure.

The Committee noted the previous assessments of exposure to AFT made by JECFA in 1998 (Annex 1, reference *131*) and EFSA in 2007 (*55*). The estimates made at the present meeting for EU dietary exposures (0.7−2.5 ng/kg bw per day for European clusters B, E and F, with MLs from 4 to 20 µg/kg for tree nuts) were in the range of those reported in the EFSA opinion, where AFT exposures ranged from 1.0 to 2.5 ng/kg bw per day (with MLs from 4 to 10 µg/kg for tree nuts, and including high-level consumers of these nuts), compared with 0.8 ng/kg bw per day reported by JECFA in 1998 (with MLs from 10 to 20 µg/kg in groundnuts). In these estimates, groundnuts and maize were the main contributors to AFT exposure, ranging from 0.2 to 1.4 ng/kg bw per day at the current meeting, compared with 1.1−2.0 ng/kg bw per day in the 1998 JECFA evaluation and 0.03−1.0 ng/kg bw per day in the EFSA opinion.

5.1.8 *Evaluation*

The Committee noted that the majority of data included in the estimation of dietary AFT exposure from foods other than almonds, Brazil nuts, hazelnuts, pistachios and dried figs came from the EU and that these data do not reflect

the actual mean values in other world regions. This probably results in an underestimate of dietary AFT exposure and overstates the relative contribution of dietary AFT exposure from tree nuts. The Committee decided to base the assessment of the impact of different MLs for AFT on data provided by producing countries, noting that these better represent the materials in commerce and result in a robust estimate of dietary AFT exposure from tree nuts.

The Committee calculated that the consumption of almonds, Brazil nuts, hazelnuts, pistachios and dried figs contributes greater than 5% of the dietary AFT exposure in only five cluster diets (clusters B, C, D, E and M). If fully enforced, an ML at 20 µg/kg in almonds, Brazil nuts, hazelnuts, pistachios and dried figs would have an impact on the relative contribution to dietary AFT exposure only in these clusters, including high-level consumers of the tree nuts. This is due solely to the elevated AFT level in pistachios. For the tree nuts other than pistachios, the presence of an ML has no effect on dietary AFT exposure.

Moreover, the Committee concluded that enforcing an ML of 15, 10, 8 or 4 µg/kg would have little further impact on the overall dietary exposure to AFT in all five of the highest exposed population groups, compared with setting an ML of 20 µg/kg. The proportion of rejected samples from the world market would be between 1% (ML 20 µg/kg) and 3% (ML 4 µg/kg) for almonds, 11% and 17% for Brazil nuts, 1% and 7% for hazelnuts and 40% and 60% for pistachios, respectively.

Based on the large data sets on AFT concentrations in dried figs submitted at this meeting by Turkey, the most important producing country of dried figs (>40 000 data points), the Committee concluded that whatever the hypothetical ML scenario applied (no ML, 4, 8, 10, 15 or 20 µg/kg) to dried figs, there would be no impact on the overall dietary exposure to AFT (below 0.03%, equivalent to a dietary exposure of <0.01 ng/kg bw per day), and that the proportion of rejected samples from the world market could range between 1% and 3% for MLs at 20 µg/kg and 4 µg/kg, respectively.

The Committee noted that the reduction of dietary AFT exposure is an important public health goal, particularly in populations that consume high levels of any potentially AFT-contaminated food.

A toxicological monograph was prepared.

5.2 Ochratoxin A

5.2.1 *Explanation*

Ochratoxin A was first evaluated by the Committee at its thirty-seventh meeting (Annex 1, reference *94*). The key adverse effects noted involved

toxicity to the kidney. The Committee established a provisional tolerable weekly intake (PTWI) of 112 ng/kg bw, on the basis of deterioration of renal function in pigs, for which the LOEL was 8 µg/kg bw per day, and application of a safety factor of 500. At that time, the Committee recommended that further studies be conducted to elucidate the role of ochratoxin A in causing nephropathy in pigs, the mode of action of ochratoxin A as a kidney carcinogen in rodents and the possible role of ochratoxin A in human disease.

Ochratoxin A was re-evaluated by the Committee at its forty-fourth meeting (Annex 1, reference *116*), when it considered toxicological data that had become available since the previous evaluation, including epidemiological studies of nephropathy, genotoxicity studies and studies on experimental nephrotoxicity. At that meeting, the Committee reconfirmed the PTWI, rounding it to 100 ng/kg bw, and reiterated its request for further studies on ochratoxin A.

Ochratoxin A was again evaluated by the Committee at its fifty-sixth meeting (Annex 1, reference *152*), when it was noted that the adverse effect at the lowest effective dose in several mammalian species was nephrotoxicity and that this was likely to be true in humans as well. Although an association between the intake of ochratoxin A and nephropathy in humans had been postulated, causality had not been established. Concerning carcinogenicity, the Committee concluded that the new toxicological data available since the previous evaluation raised further questions about the mode of action of ochratoxin A. Both genotoxic and non-genotoxic modes of action had been proposed. The Committee noted that further studies to address these issues were in progress and retained the previously established PTWI of 100 ng/kg bw, pending the results of these studies.

New data from surveys of food commodities for contamination with ochratoxin A were also considered, and intakes were estimated for various countries and regions of the world. From estimates based mainly on European data, the Committee noted that the intake of ochratoxin A by 95th percentile consumers of cereals may approach the PTWI from this source alone. Given the distribution of contamination of cereals with ochratoxin A, the Committee concluded that application of an ML of 5 or 20 µg/kg would make no significant difference to the average intake. Efforts were needed to ensure that intakes of ochratoxin A did not exceed the PTWI, and the Committee considered that this could best be achieved by lowering overall contamination by appropriate agricultural, storage and processing practices.

At the thirty-eighth session of CCFAC (*7*), the Committee was again asked to re-evaluate ochratoxin A, considering all the data available on toxicology and exposure assessment, particularly data from developing countries, in-

cluding the impact of different MLs for cereals (5 or 20 μg/kg) and effects of processing on residual levels in foods.

For this evaluation, the Committee considered new toxicological studies that had become available since the last evaluation; these included further studies on developmental toxicity, neurotoxicity, immunotoxicity, nephrotoxicity and genotoxicity and studies on the mode of action of ochratoxin A in the kidney. The Committee also considered the opinion on ochratoxin A in human food published by EFSA in 2006 (*56*). New data on analytical methods, sampling protocols and the effects of processing were also considered, together with methods of prevention and control and levels and patterns of food contamination. A new dietary exposure assessment was conducted, and the impact of different MLs for cereals was considered.

5.2.2 *Absorption, distribution, metabolism and excretion*

Ochratoxin A is efficiently absorbed from the gastrointestinal tract, mainly in the small intestine. Information from a number of species shows that it is distributed via the blood mainly to the kidneys, with lower concentrations found in liver, muscle and fat. Specific transporters may be involved in the cellular uptake of ochratoxin A into the kidney, where it accumulates. Transfer to milk has been demonstrated in rats, rabbits and humans, but little ochratoxin A is transferred to the milk of ruminants, owing to efficient hydrolysis of the amide bond by microflora in the rumen, to yield phenylalanine and ochratoxin *alpha*. Ochratoxin *alpha*, a chlorinated dihydroisocoumarin, is the major metabolite of ochratoxin A in all species examined. This and minor hydroxylated metabolites of ochratoxin A that have been identified are all reported to be less toxic than ochratoxin A itself. Ochratoxin A is excreted in urine and faeces, and the relative contribution of each of these routes in different species is influenced by the extent of enterohepatic recirculation of ochratoxin A and its binding to serum proteins. These factors are also important in the determination of the serum half-life of ochratoxin A, which varies widely among species. Ochratoxin A has a long half-life in some non-ruminant mammals, ranging from 1−1.5 days in mice, 2−5 days in rats and 3−5 days in pigs up to around 20 days in macaque and vervet monkeys and 35 days in a human volunteer.

5.2.3 *Toxicological data*

None of the new studies on nephrotoxicity, developmental toxicity, neurotoxicity or immunotoxicity that have appeared since the Committee's last evaluation would have an impact on the Committee's previous selection of minimal renal changes in the pig, observed at a dose of 8 μg/kg bw per day (the LOEL), as a critical effect for risk assessment.

In its previous evaluation, the Committee commented that the mechanism by which ochratoxin A causes renal tumours was unknown, noting that both genotoxic and non-genotoxic (epigenetic) modes of action had been proposed. Investigation of the mode of action of ochratoxin A in the kidney, with particular reference to carcinogenic effects, has been a key driver of much of the research conducted since then.

Several hypotheses on the mode of action of ochratoxin A as a carcinogen have been proposed, and evidence has been generated in support of each of them. Some of these would completely account for tumour formation, whereas others have been considered as possible contributors to tumour formation. They can be summarized as follows:

- genotoxicity from direct interaction of ochratoxin A or a reactive metabolite with DNA;
- generation of tumours secondary to chronic renal toxicity and compensatory cell proliferation;
- generation of tumours secondary to inhibition of phenylalanine–tRNAPhe synthetase and protein synthesis;
- disruption of cell–cell signalling pathways and the process of cell division;
- alteration of intracellular calcium homeostasis;
- mitochondrial dysfunction leading to oxidative stress and indirect induction of DNA damage.

Concerning a genotoxic mode of action, divergent results have been obtained in the large number of genotoxicity assays on ochratoxin A, most of which were available at the time of the Committee's previous evaluation. In more recent comet assays, there was evidence of DNA damage in vitro and in vivo, including in rat kidney. However, these positive results were generally obtained with high ochratoxin A exposure levels and, where investigated, were indicative of oxidative damage. The Committee noted that whereas some investigators have previously reported formation of a number of different DNA adducts detectable by the ^{32}P-postlabelling technique under different in vitro and in vivo conditions, particularly following prolonged, high exposures to ochratoxin A, others have not been able to detect DNA adduct formation, despite, in some cases, using similar doses of ochratoxin A and more sensitive techniques. The Committee concluded that a direct genotoxic mode of action, by demonstration of covalent binding to DNA with the formation of DNA adducts containing ochratoxin A or a metabolite of ochratoxin A, has not been confirmed.

Concerning non-genotoxic modes of action, a number of recent studies have addressed early changes associated with ochratoxin A exposure in vitro and in vivo, including indicators of oxidative stress, alterations in gene expression and cell signalling pathways, increased apoptosis, disruption of cell mitosis and increases in cell proliferation. Recent work has shown marked dose- and time-related increases in renal cell proliferation after 4 and 13 weeks of gavage administration of ochratoxin A to rats at the same doses that gave rise to renal tumour formation in the 2-year rat study (70 and 210 μg/kg bw per day, 5 days/week), with a NOAEL for cell proliferation at the same dose as that where no increase in tumour formation was observed in the 2-year rat study (21 μg/kg bw per day, 5 days/week). The Committee noted that cell proliferation, which is known to be effective in increasing tumour incidence via conversion of DNA damage into permanent mutations, may be a key event in the mechanism of tumour formation with ochratoxin A. Overall, the Committee considered that the evidence points to a number of non-genotoxic modes of action that could plausibly be involved in the generation of renal tumours, and this supports the previous decision to set a PTWI.

In order to provide additional information for the risk assessment, the Committee performed BMD modelling using the carcinogenicity data on ochratoxin A from the rat bioassay performed by the United States National Toxicology Program in 1989 (*57*). The combined adenoma and carcinoma data from male rat kidney, representing the most sensitive sex, species and target organ for ochratoxin A carcinogenicity, were used for modelling. Six different models were fitted to the dose–incidence data to estimate the lower limits of one-sided 95% confidence intervals on the BMD representing a 10% renal tumour incidence ($BMDL_{10}$s). The lowest $BMDL_{10}$ had a value of 15 μg/kg bw per day, 5 days/week, and the model showing the best fit had a value of 25 μg/kg bw per day, 5 days/week. Thus, for establishing the PTWI, the $BMDL_{10}$ does not provide a lower point of departure than the LOEL of 8 μg/kg bw per day for minimal renal toxicity changes in the pig.

5.2.4 *Observations in humans*

The earlier literature on the association between human exposure to ochratoxin A and the occurrence of Balkan endemic nephropathy and associated urinary tract tumours was summarized in the previous evaluation (Annex 1, reference *153*). Contrary to the clear causal evidence of ochratoxin A-induced nephrotoxicity and kidney carcinogenicity in rodents, the significance of ochratoxin A for human health remains unclear from the available epidemiological evidence. Moreover, ochratoxin A exposure is only one of several hypotheses concerning an environmental etiology for Balkan endemic nephropathy.

Blood concentration of ochratoxin A appears to be a reliable biomarker of exposure in humans. In the Committee's previous evaluation (Annex 1, reference *153*), the concentrations of ochratoxin A in blood samples from healthy persons, obtained in surveys conducted in 17, mainly European, countries, ranged between 0.1 and 40 ng/ml (with an exceptional maximum of 160 ng/ml). The concentrations of ochratoxin A in blood samples obtained in more recent surveys from nine countries, four of which are European, ranged between 0.15 and 1.14 ng/ml, suggesting a possible decline in extreme peak values for blood concentrations compared with earlier surveys.

5.2.5 *Analytical methods*

Methods for analysing ochratoxin A were thoroughly reviewed in the previous report (Annex 1, reference *153*). At that time, validated analytical methods were already available for the determination of ochratoxin A in maize, barley, rye, wheat, wheat bran, wholemeal wheat, roasted coffee, wine and beer. The best methods used liquid chromatography following cleanup using immunoaffinity columns. Recent developments in analytical methodology are described in the addendum to the monograph (Annex 1, reference *188*).

5.2.6 *Sampling protocols*

The only recent development in this area has been the publication of extensive information on the parameters governing sample size for testing green coffee for ochratoxin A.

5.2.7 *Fungi producing ochratoxin A*

At the fifty-sixth meeting of JECFA (Annex 1, reference *152*), it was reported that ochratoxin A is produced by three taxonomically distinct groups of fungi: a single *Penicillium* species, *P. verrucosum*; *Aspergillus ochraceus* and several related *Aspergillus* species; and *A. carbonarius*, with a small percentage of isolates of the closely related species *A. niger*. Since that report, some other *Aspergillus* and *Penicillium* species have been described as potential sources of ochratoxin A. Details of the revised taxonomy of the fungi producing ochratoxin A and of the physiology and ecology of these fungi are described in the addendum to the monograph (Annex 1, reference *188*).

5.2.8 *Effects of processing*

A few recent studies have examined the effect of processing on concentrations of ochratoxin A in wine, coffee and European cereals.

Wine

Vinification has consistently been reported to reduce ochratoxin A concentrations in wine, independent of the initial ochratoxin A concentration in grapes. Ochratoxin A is removed at each solid–liquid separation stage of the process.

Coffee

There is general agreement that roasting has an influence on ochratoxin A concentrations, but the percentage reduction reported has varied widely. Light roasting causes reductions in ochratoxin A of 0–80%. Dark roasting (i.e. to a typical espresso coffee) may cause reductions of more than 90% in ochratoxin A content.

Milling and breadmaking

Using whole wheat contaminated with ochratoxin A, a combination of cleaning, scouring, removal of the bran fraction and baking caused an overall reduction of about 75% of ochratoxin A in white bread.

Extrusion

The extrusion of wholemeal wheat contaminated with ochratoxin A resulted in a reduction of no more than 40% of the toxin, even under the harshest conditions likely to be used in commercial practice.

5.2.9 *Prevention and control*

The principal fungi that produce ochratoxin A in foods—*Aspergillus carbonarius*, *A. westerdijkiae*, *A. steynii*, *Penicillium verrucosum* and *P. nordicum*—are not associated with plants and hence are not usually present in food crops before harvest. The control of ochratoxin A in foods, therefore, is basically a post-harvest problem. The basic concepts of good harvest practice, of drying crops rapidly and keeping them dry in storage, transport and processing systems, will ensure that crops remain essentially free of ochratoxin A. One exception is the entry of *A. carbonarius* into grapes before harvest.

Grapes, wine and other grape products

Once *A. carbonarius* has gained entry to a grape via damaged skin, the high sugar/high acid combination provides a perfect medium for ochratoxin A production. The keys to low ochratoxin A levels in wine are the reliance on prevention of infection of grapes by pathogens, rapid harvest if rain causes skin splitting, good harvest practice, including rejection of poor quality

bunches, and a minimal delay between harvest and crushing. Control of ochratoxin A formation in dried vine fruits is less easy, because any pre-harvest infection with *A. carbonarius* will continue to develop during the early stages of drying.

Coffee

Aspergillus westerdijkiae, *A. steynii* and *A. carbonarius* are the major causes of ochratoxin production in green coffee. Infection of coffee cherries occurs during handling after harvest and in the drying yard. Many coffee-growing areas in the world are subjected to misty or rainy conditions after harvest, with consequent slow drying of the coffee cherries and ochratoxin A formation. However, good processing and drying regimes can prevent this problem.

Cereals

Where ochratoxin A occurs in cool temperate zone cereals, it is produced by the growth of *P. verrucosum.* Again, there is no evidence that this species infects growing cereal plants or occurs in nature in grains before harvest. The key to controlling ochratoxin A in cereals is rapid drying; however, in cool temperate zones, grain is often harvested during moist or rainy conditions, and rapid drying may be difficult in practice.

There were limited data available to the Committee on cereals grown in tropical zones, but ochratoxin A has been found to occur in sorghum, maize and millet.

Meat products

If meat products are infected by *P. nordicum*, this occurs during the processing stages. Control of levels of fungal spores in the air will reduce this problem.

5.2.10 *Dietary exposure assessment*

Analysis of data submitted

Data on occurrence of ochratoxin A in cereals were submitted by Canada, Germany, Japan and the EU. In addition, data reporting the contamination of cereals in Nigeria, Ghana and Burkina Faso were submitted by FAO, and information regarding the contamination of cocoa and coffee beans was submitted by Côte d'Ivoire. Table 17 describes the various aspects of the distribution of ochratoxin A contamination in cereals. The most contaminated commodities in the German data set were rye and buckwheat, whereas the most contaminated commodities in the African data set were sorghum, maize and milletons.

Table 17
Description of data submitted for this evaluation: distribution of ochratoxin A contamination in cereals

Countries	Number of samples	Number of non-detected[a] (%)	Samples >LOD or LOQ and <5 µg/kg (%)	Samples ≥5 µg/kg (%)	Samples >10 µg/kg (%)	Samples >15 µg/kg (%)	Samples >20 µg/kg (%)
Germany (RC)	1696	1428 (84)	250 (15)	18 (1.1)	8 (0.5)	6 (0.4)	6 (0.4)
Germany (PC)	2377	1717 (72)	639 (27)	21 (0.9)	11 (0.5)	5 (0.2)	2 (0.1)
Japan	197	188 (95)	9 (5)	0	0	0	0
Africa	27	9 (33)	16 (59)	2	0	0	0
Canada (RC)	1016	827 (82)	–	–	–	–	–
Canada (PC)	652	405 (62)	–	–	–	–	–
EU (RC + PC)	5117	2295 (45)	2779 (54)	43 (0.8)	17 (0.3)	–	10 (0.2)

RC, raw cereals; PC, processed cereals.
[a] Non-detected samples are those for which no numerical value was provided.

Estimation of the concentration of ochratoxin A in cereals

The critical effects of ochratoxin A relate to long-term exposure, and therefore the central tendency of the distribution of contamination should be used for dietary exposure assessments. Owing to the fact that generally more than 50% of analysed samples are below the LOD or LOQ, the use of the median value to represent this central tendency is problematic. Therefore, the international exposure assessments to date are based on the mean level of contamination.

The data from Africa were not used for the dietary exposure assessment because they were targeted samples with fungal contamination. Individual data were not available from either Canada or the EU. Finally, the Committee decided not to merge individual analytical data from Germany and Japan, but to focus the dietary exposure assessment on the largest data set of analytical results for raw and processed cereals from Germany.

Impact of left and right censorship on average level of contamination

Information on LOD and LOQ is essential for the purpose of the current assessment to assign values to non-detect (ND) results. The assignment of ND equal to 0 and ND equal to LOD or LOQ was used to provide a lower- and an upper-bound concentration of ochratoxin A in cereals.

Analytical results (detected or non-detected) for which information on LOD or LOQ was not available were removed from the data set. In addition, because available LOD or LOQ values were very variable, the impact of removing analytical data obtained with the highest LOQs was tested. The Committee excluded all samples (detected and non-detected) where the LOQ was higher than 1 µg/kg because the method of analysis was not very sensitive and there were sufficient data for the analysis obtained by more sensitive methods.

From the available data, it is possible to simulate the impact of various MLs, assuming 100% enforcement, on mean ochratoxin A concentration by excluding analytical results above each ML. Based on the available individual analytical results for raw cereals from Germany, the mean contamination was calculated using two scenarios for left censorship (i.e. ND = 0 and ND = LOD or LOQ), two scenarios for right censorship (i.e. ML at 5 or 20 µg/kg) and finally a scenario without an ML, resulting in six scenarios compiled in Table 18.

Table 18
Ochratoxin A distribution in the German data set on raw cereals (1462 samples): impact of various MLs on the mean concentration of ochratoxin A

	Concentration of ochratoxin A in raw cereals (µg/kg)	
	ND = 0	ND = LOD or LOQ
Distribution of ochratoxin A		
Mean	0.44	0.53
Median	0.00	0.10
90th percentile	0.26	0.50
95th percentile	0.78	0.90
99th percentile	4.98	4.98
Maximum	125.00	125.00
Impact of MLs on mean concentration of ochratoxin A		
ML = 5 µg/kg	0.10	0.19
ML = 20 µg/kg	0.15	0.24
No ML	0.44	0.53

The available data show a limited impact of the various MLs on the mean contamination of raw cereals by ochratoxin A. However, it is important to note that the observed differences are due to very few high analytical results (15 samples out of 1462 excluded when the ML is 5 µg/kg and 6 samples out of 1462 excluded when the ML is 20 µg/kg).

Impact of new data on estimates of dietary exposure to ochratoxin A

In 2001, the mean overall dietary exposure to ochratoxin A, based mainly on European data, was estimated by the Committee to be 43 ng/kg bw per week (Annex 1, reference *152*). The contribution from cereals was estimated to be 58% of the overall exposure (25 ng/kg bw per week), based on a daily consumption of 230 g of cereals and a mean ochratoxin A concentration of 0.94 µg/kg for raw cereals.

At the present meeting, mean concentrations of ochratoxin A for processed cereals were estimated to be 0.31 µg/kg for the lower bound and 0.39 µg/kg for the upper bound, based on German data (Table 19). In order to perform a realistic estimate of dietary exposure from the consumption of cereals, the Committee decided to consider both the mean consumption of cereals from the five GEMS regional diets (230 g in Europe) and the mean consumption of cereals from the 13 GEMS/Food Consumption Cluster Diets (365 g in cluster E, includes Germany). The resulting dietary exposure estimate ranged from 8 to 17 ng/kg bw per week, which can be compared with the previous estimate of 25 ng/kg bw per week.

Table 19
Description of ochratoxin A distribution in the German data set on processed cereals (2070 samples): impact of various MLs on the mean concentration of ochratoxin A

	Concentration of ochratoxin A in processed cereals (μg/kg)	
Distribution of ochratoxin A	ND = 0	ND = LOD or LOQ
Mean	0.31	0.39
Median	0.00	0.10
90th percentile	0.65	0.70
95th percentile	1.40	1.40
99th percentile	4.73	4.73
Maximum	22.80	22.80

5.2.11 ***Evaluation***

The new data, including data on mode of action of ochratoxin A in the kidney, do not indicate any reason to modify the previous approach taken by JECFA with respect to setting a PTWI. The Committee therefore retained the previous PTWI of 100 ng/kg bw.

The current estimate of overall dietary exposure to ochratoxin A from cereals, based mainly on European data, is about 8−17 ng/kg bw per week, based on processed cereals, compared with 25 ng/kg bw per week in the previous evaluation, based on raw cereals. The current estimate is well below the PTWI.

Contamination levels in the majority of raw cereal samples were below 5 μg/kg. Owing to the very small number of samples contaminated above the highest proposed limit of 20 μg/kg for cereals, such an ML would have very limited impact compared with no ML. The Committee concluded that the use of an ML of 5 or 20 μg/kg would be unlikely to have an impact on dietary exposure to ochratoxin A. The Committee was unable to reach a conclusion regarding the situation in developing countries, owing to the lack of adequate data with which to do an assessment.

An addendum to the toxicological monograph was prepared.

6. Future work

- The Committee agreed to repeat the assessment of a selected number of flavouring agents using both the MSDI and SPET dietary exposure estimates for evaluation at the next meeting.
- The Committee agreed to further develop suitable criteria for selecting flavouring agents where additional information on added use levels recommended by the industry is required for use in the SPET, prior to evaluation.
- The Committee recommended that the assessment of combined dietary exposure for flavouring agents at future meetings should be undertaken for flavouring agents that share a common metabolite and for flavouring agents that are members of a homologous series.
- The Committee recommended that future evaluations adopt a stepwise procedure developed at this meeting to determine whether a new product might be included in a previously allocated ADI.
- The Committee recommended that the subject of guidelines for the safety evaluation of enzymes produced by GMMs be addressed at a future meeting.
- Pursuant to its decision that substances with food additive uses in addition to flavouring agent use should have specifications in both food additives and flavouring agent format and that each should contain comparable purity requirements, the Committee agreed to review at a future meeting whether any substantial differences exist between specifications in the food additives format with flavouring agent as a functional use and the flavouring agent monograph for the same substance.
- The Committee noted that the previous dietary exposure estimate for carrageenan was made solely using production poundage and may be outdated. The Committee therefore recommended that a new dietary exposure evaluation, employing specific food type and use level information, be undertaken, ensuring that new uses are adequately taken into consideration.

7. Recommendations

1. The Committee noted that the test method for dimethyl sulfoxide in the specifications for sucrose esters of fatty acids still used an outdated GC column and recommended that further efforts be taken to find a suitable replacement.

2. In its discussions on AFL, the Committee noted the following:

   - Surveillance data should be accompanied by a clear description of the analytical method used, recoveries of the analytical methodology chosen should be specific to the food matrix tested, and LODs and LOQs should be provided with the definitions used to derive them.
   - Efforts should be made to harmonize the nomenclature and the methodologies by which the LOD and LOQ are calculated.
   - There remains a need for harmonized sampling plans, both between different countries and within the same country.

Acknowledgements

The Committee learned with sadness of the passing of Professor Robert Kroes. Professor Kroes was a frequent member of JECFA, making a very significant contribution to the work of the Committee. Bobby, as he was known to all with whom he worked, was a leading scientific authority in toxicology, with broad expertise, including pathology and related disciplines. He was strongly influential in the development of the TTC concept used by the Committee for the safety evaluation of flavours and published several key papers concerning the application of TTC in the risk assessment framework. Professor Kroes will be remembered for his wise counsel, depth of knowledge and, most importantly, his common sense approach to complex problems. Bobby will also be greatly missed by his friends and colleagues, all of whom enjoyed his warmth and tremendous sense of humour.

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Annex 1

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

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2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. I. *Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. II. *Food colours*, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
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187. *Evaluation of certain food additives and contaminants* (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.

188. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 59 (in preparation).

189. *Compendium of food additive specifications.* FAO JECFA Monographs 4 (in preparation).

Annex 2

Acceptable daily intakes, other toxicological information and information on specifications

Food additives and ingredients evaluated toxicologically or assessed for dietary exposure

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Acidified sodium chlorite (ASC)		The available toxicological data were sufficient to assess the safety of ASC by setting ADIs for chlorite and chlorate. Chlorite: ADI of 0–0.03 mg/kg bw Chlorate: ADI of 0–0.01 mg/kg bw New specifications were prepared for sodium chlorite and one of the acids used in the preparation of ASC, sodium hydrogen sulfate.
Asparaginase from *Aspergillus oryzae* expressed in *Aspergillus oryzae*	N	ADI "not specified"[b] when used in the applications specified and in accordance with good manufacturing practice.
Carrageenan and processed *Eucheuma* seaweed	R R	The group ADI "not specified"[b] for the sum of carrageenan and processed *Eucheuma* seaweed was maintained for food additive uses in foods other than infant formula. The Committee was of the view that based on the information available, it is inadvisable to use carrageenan or processed *Eucheuma* seaweed in infant formulas.
Cyclotetraglucose and cyclotetraglucose syrup (listed on draft agenda as cyclotetraose)	N N,T	A temporary ADI "not specified"[b] was allocated for cyclotetraglucose and cyclotetraglucose syrup pending submission of data on the identity of the bacterial strain used to produce the 6-GT/IMT enzyme preparation and evidence of its lack of pathogenicity and toxigenicity. The specifications for cyclotetraglucose syrup were made tentative pending information on the total saccharide content and test methods and the unidentified fraction.

Food additive	Specifications[a]	Acceptable daily intake (ADI) and other toxicological recommendations
Isoamylase from *Pseudomonas amyloderamosa*	N	ADI "not specified"[b] when used in the applications specified and in accordance with good manufacturing practice.
Magnesium sulfate	R	ADI "not specified"[b]
Phospholipase A1 from *Fusarium venenatum* produced by *Aspergillus oryzae*	S	ADI "not specified"[b] when used in the applications specified and in accordance with good manufacturing practice.
Sodium iron(III) ethylenediaminetetraacetic acid (EDTA)	S	Sodium iron EDTA is suitable for use as a source of iron for food fortification to fulfil nutritional iron requirements, provided that the total intake of iron from all food sources including contaminants does not exceed the PMTDI of 0.8 mg/kg bw. Total intake of EDTA should not exceed acceptable levels, also taking into account the intake of EDTA from the food additive use of other EDTA compounds. An ADI of 0–2.5 mg/kg bw was previously established for the calcium disodium and disodium salts of EDTA, equivalent to up to 1.9 mg EDTA/kg bw.
Steviol glycosides	R	The temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, was extended until 2008, pending submission of the results of the ongoing studies. The Committee considered that the newly available data did not raise additional concerns regarding the safety of steviol glycosides, but that the results of ongoing clinical studies, which more closely address the requirements specified at the sixty-third meeting, would be essential to its evaluation. The specifications were revised and the tentative assignation was removed. The method of assay includes a minimum requirement of 95% of the total of seven steviol glycosides.

[a] N: new specifications prepared; R: existing specifications revised; S: existing specifications maintained; 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.

Food additives, including flavouring agents, considered for specifications only

Food additive	Specifications[a]
Anisyl acetone	W
Furfural	W
Ethyl maltol	R
Maltol	R
Nisin preparation	R
Pectins	R
Polyvinyl alcohol	R
Sucrose esters of fatty acids	R
Zeaxanthin-rich extract from *Tagetes erecta*	W

Flavouring agent	JECFA No.	Specifications[a]
3-Acetyl-2,5-dimethylfuran	1506	R
Ethyl maltol	1481	R
Maltol	1480	R
Maltyl isobutyrate	1482	R
3-Methyl-2-oxobutanoic acid	631	R
3-Methyl-2-oxopentanoic acid	632	R
4-Methyl-2-oxopentanoic acid	633	R
Sodium 3-methyl-2-oxobutanoate	631.1	R
Sodium 3-methyl-2-oxopentanoate	632.1	R
Sodium 4-methyl-2-oxopentanoate	633.1	R
Sodium 2-oxo-3-phenylpropionate	1479	R
2,4,5-Trimethyl-delta-oxazolin	1559	R

[a] R: existing specifications revised; W: existing specifications withdrawn.

Food contaminants evaluated toxicologically or assessed for dietary exposure

Food contaminant	Tolerable intakes and other toxicological recommendations
Aflatoxins (AFL) (Intake assessment from almonds, Brazil nuts, hazelnuts, pistachios and dried figs, impact of various MLs)	The Committee decided to base the assessment of the impact of different MLs for AFL exposure on data provided by producing countries, noting that these better represent the materials in commerce and result in a robust estimate of AFL dietary exposure from the tree nuts. Consumption of almonds, Brazil nuts, hazelnuts, pistachios and dried figs contributes greater than 5% of the total AFL dietary exposure in only 5 of the 13 GEMS/Food Consumption Cluster Diets (clusters B, C, D, E and M). If fully enforced, an ML at 20 µg/kg in almonds, Brazil nuts, hazelnuts, pistachios and dried figs would have an impact on the relative contribution to dietary AFL exposure only in these clusters, including high-level consumers of tree nuts. This

Food contaminant	Tolerable intakes and other toxicological recommendations
	contribution is due solely to the elevated AFL level in pistachios. For tree nuts other than pistachios, the presence of an ML has no effect on dietary AFL exposure. Moreover, the Committee concluded that enforcing an ML of 15, 10, 8 or 4 µg/kg would have little further impact on the overall dietary exposure to AFL in all five of the highest exposed population groups compared with setting an ML of 20 µg/kg. Regarding dried figs, the Committee concluded that whatever the hypothetical ML scenario applied (no ML, 4, 8, 10, 15 or 20 µg/kg), there would be no impact on the overall dietary exposure to AFL. The Committee noted that the reduction of dietary AFL exposure is an important public health goal, particularly in populations that consume high levels of any potentially AFL-contaminated food.
Ochratoxin A	The previous PTWI of 100 ng/kg bw was retained. The new data, including data on mode of action of ochratoxin A in the kidney, do not indicate any reason to modify the previous risk assessment approach taken by JECFA. The current estimate of overall dietary exposure to ochratoxin A from cereals, based mainly on European data, is about 8–17 ng/kg bw per week, based on processed cereals, compared with 25 ng/kg bw per week in the previous evaluation, based on raw cereals. The current estimates are well below the PTWI. Contamination levels in the majority of raw cereal samples were below 5 µg/kg. Owing to the very small number of samples contaminated above the highest proposed limit of 20 µg/kg, such an ML would have very limited impact compared with no ML. The Committee concluded that the use of an ML of 5 or 20 µg/kg would be unlikely to have an impact on dietary exposure to ochratoxin A. The Committee was unable to reach a conclusion regarding the situation in developing countries, owing to the lack of adequate data to consider.

Flavouring agents evaluated using the Procedure for the Safety Evaluation of Flavouring Agents

A. Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Ethyl-2-methyl-3,4-pentadienoate	353	S	No safety concern
Methyl 4-pentenoate	1616	N	No safety concern
2-Methylbut-2-en-1-ol	1617	N	No safety concern
Ethyl 4-pentenoate	1618	N	No safety concern
4-Pentenal	1619	N	No safety concern
3-Isopropenylpentanedioic acid	1620	N	No safety concern
trans-3-Hexenol	1621	N	No safety concern
trans-4-Hexenal	1622	N	No safety concern
5-Hexenol	1623	N	No safety concern

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Methyl (*Z*)-3-hexenoate	1624	N	No safety concern
cis-4-Octenol	1625	N	No safety concern
Ethyl (*Z*)-3-hexenoate	1626	N	No safety concern
3-Octenoic acid	1627	N	No safety concern
(*Z*)-3-Octenyl propionate	1628	N	No safety concern
trans-4-Octenoic acid	1629	N	No safety concern
Methyl (*Z*)-5-octenoate	1630	N	No safety concern
cis-5-Octenoic acid	1631	N	No safety concern
Ethyl 3-octenoate	1632	N	No safety concern
cis-4-Decenol	1633	N	No safety concern
Isobutyl 10-undecenoate	1634	N	No safety concern
11-Dodecenoic acid	1635	N	No safety concern
(*Z*)-4-Dodecenal	1636	N	No safety concern
cis-9-Octadecenol	1637	N	No safety concern
cis-9-Octadecenyl acetate	1638	N	No safety concern
Methyl 10-undecenoate	1639	N	No safety concern
(*Z*)-8-Tetradecenal	1640	N	No safety concern
9-Octadecenal	1641	N	No safety concern
(*E*)-4-Nonenal	1642	N	No safety concern

[a] N: new specifications prepared; S: existing specifications maintained.

B. Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
2,3,4-Trimethyl-3-pentanol	1643	N	No safety concern
(±)-2,4,8-Trimethyl-7-nonen-2-ol	1644	N	No safety concern
(*E*)- and (*Z*)-2,4,8-Trimethyl-3,7-nonadien-2-ol	1645	N	No safety concern
Nerolidol	1646	N	No safety concern
1-Phenyl-3-methyl-3-pentanol	1649	N	No safety concern
p-α,α-Trimethylbenzyl alcohol	1650	N	No safety concern
(±)-Ethyl 2-hydroxy-2-methylbutyrate	1651	N	No safety concern
(±)-Ethyl 2-hydroxy-3-methylvalerate	1652	N	No safety concern
α,α-Dimethylphenethyl alcohol	1653	N	No safety concern
α,α-Dimethylphenethyl formate	1654	N	No safety concern
α,α-Dimethylphenethyl acetate	1655	N	No safety concern
α,α-Dimethylphenethyl butyrate	1656	N	No safety concern
α,α-Dimethylbenzyl isobutyrate	1657	N	No safety concern
Structural class II			
6-Acetoxydihydrotheaspirane	1647	N	No safety concern
6-Hydroxydihydrotheaspirane	1648	N	No safety concern

[a] N: new specifications prepared.

C. Simple aliphatic and aromatic sulfides and thiols

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Simple sulfides			
Structural class I			
2-Methyl-1-methylthio-2-butene	1683	N	No safety concern
2,4,6-Trithiaheptane	1684	N	No safety concern
2,5-Dithiahexane	1707	N	No safety concern
Acyclic sulfides with oxidized and thiol side-chains			
Structural class I			
Methionyl butyrate	1668	N	No safety concern
Methylthiomethylmercaptan	1675	N	No safety concern
(±)-Isobutyl 3-methylthiobutyrate	1677	N	No safety concern
3-(Methylthio)-2-butanone	1688	N	No safety concern
4-(Methylthio)-2-pentanone	1689	N	No safety concern
Methyl 3-(methylthio)butanoate	1690	N	No safety concern
Methyl (methylthio)acetate	1691	N	No safety concern
(±)-3-(Methylthio)heptanal	1692	N	No safety concern
(±)-3-(Ethylthio)butanol	1703	N	No safety concern
S-Allyl-L-cysteine	1710	N	No safety concern
Heterocyclic sulfides			
Structural class I			
(±)-2,8-Epithio-*cis*-*p*-menthane	1685	N	No safety concern
Simple thiols			
Structural class I			
Ethanethiol	1659	N	No safety concern
1-Pentanethiol	1662	N	No safety concern
Heptane-1-thiol	1663	N	No safety concern
2-Heptanethiol	1664	N	No safety concern
Structural class II			
(±)-1-Phenylethylmercaptan	1665	N	No safety concern
Thiols with oxidized side-chains			
Structural class I			
Propyl 2-mercaptopropionate	1667	N	No safety concern
(±)-4-Mercapto-4-methyl-2-pentanol	1669	N	No safety concern
4-Mercapto-2-pentanone	1670	N	No safety concern
(*S*)-1-Methoxy-3-heptanethiol	1671	N	No safety concern
Methyl 3-mercaptobutanoate	1674	N	No safety concern
Hexyl 3-mercaptobutanoate	1704	N	No safety concern
(±)-3-Mercapto-1-butyl acetate	1705	N	No safety concern
3-Mercapto-3-methyl-1-butyl acetate	1706	N	No safety concern
3-Mercaptoheptyl acetate	1708	N	No safety concern
Structural class II			
cis- and *trans*-Mercapto-*p*-menthan-3-one	1673	N	No safety concern
Structural class III			
2-Mercaptoanisole	1666	N	No safety concern
Diisopentyl thiomalate	1672	N	No safety concern

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Dithiols			
Structural class I			
Ethane-1,1-dithiol	1660	N	No safety concern
Dimercaptomethane	1661	N	No safety concern
bis(1-Mercaptopropyl)sulfide	1709	N	No safety concern
Simple disulfides			
Structural class I			
Ethyl methyl disulfide	1693	N	No safety concern
Ethyl propyl disulfide	1694	N	No safety concern
Methyl isopentyl disulfide	1696	N	No safety concern
Amyl methyl disulfide	1697	N	No safety concern
Butyl ethyl disulfide	1698	N	No safety concern
Diethyl disulfide	1699	N	No safety concern
Structural class II			
Allyl propyl disulfide	1700	N	No safety concern
Trisulfides			
Structural class I			
Ethyl propyl trisulfide	1695	N	No safety concern
Diethyl trisulfide	1701	N	No safety concern
Heterocyclic disulfides			
Structural class II			
3,5-Diethyl-1,2,4-trithiolane	1686	N	No safety concern
Mixture of 3,6-diethyl-1,2,4,5-tetrathiane (approx. 55%) and 3,5-diethyl-1,2,4-trithiolane (approx. 45%)	1687	N	No safety concern
Thioesters and acids			
Structural class I			
Thioacetic acid	1676	N	No safety concern
(*S*)-Methyl propanethioate	1678	N	No safety concern
(*S*)-Isopropyl 3-methylbut-2-enethioate	1679	N	No safety concern
Structural class II			
Allyl thiohexanoate	1681	N	No safety concern
Structural class III			
(*S*)-Ethyl 2-acetylaminoethanethioate	1680	N	No safety concern
Propyl propane thiosulfonate	1702	N	No safety concern

[a] N: new specifications prepared.

D. Aliphatic acyclic diols, triols and related substances

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
Dihydroxyacetone dimer	1716	N	No safety concern
1-Hydroxy-2-butanone	1717	N	No safety concern
Ethyl 3-acetoxy-2-methylbutyrate	1718	N	No safety concern
Methyl 5-acetoxyhexanoate	1719	N	No safety concern
Structural class III			
2,4-Dimethyl-1,3-dioxolane	1711	N	No safety concern
2-Hexyl-4,5-dimethyl-1,3-dioxolane	1712	N	No safety concern
cis- and *trans*-Ethyl 2,4-dimethyl-1,3-dioxolane-2-acetate	1715	N	No safety concern

[a] N: new specifications prepared.

E. Aliphatic acetals

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
(±)-1-Acetoxy-1-ethoxyethane	1726	N	No safety concern
Acetaldehyde hexyl isoamyl acetal	1727	N	No safety concern
1,1-Dimethoxy-*trans*-2-hexene	1728	N	No safety concern
Acetaldehyde diisoamyl acetal	1729	N	No safety concern
Isovaleraldehyde diethyl acetal	1730	N	No safety concern
Valeraldehyde dibutyl acetal	1731	N	No safety concern
Hexanal hexyl isoamyl acetal	1735	N	No safety concern
Hexanal dihexyl acetal	1738	N	No safety concern
Nonanal dimethyl acetal	1742	N	No safety concern
Dodecanal dimethyl acetal	1746	N	No safety concern
Acetaldehyde di-*cis*-3-hexenyl acetal	1747	N	No safety concern
Structural class III			
Isovaleraldehyde propyleneglycol acetal	1732	N	No safety concern
Isovaleraldehyde glyceryl acetal	1733	N	No safety concern
Valeraldehyde propyleneglycol acetal	1734	N	No safety concern
Hexanal octane-1,3-diol acetal	1736	N	No safety concern
Hexanal butane-2,3-diol acetal	1737	N	No safety concern
Heptanal propyleneglycol acetal	1739	N	No safety concern
2,6-Dimethyl-5-heptenal propyleneglycol acetal	1740	N	No safety concern
Octanal propyleneglycol acetal	1741	N	No safety concern
Nonanal propyleneglycol acetal	1743	N	No safety concern
Decanal propyleneglycol acetal	1744	N	No safety concern

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Undecanal propyleneglycol acetal	1745	N	No safety concern
Isobutanal propyleneglycol acetal	1748	N	No safety concern
Acetaldehyde 1,3-octanediol acetal	1749	N	No safety concern

[a] N: new specifications prepared.

F. Sulfur-containing heterocyclic compounds

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Structural class II			
1-(3-Hydroxy-5-methyl-2-thienyl)ethanone	1750	N	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl formate	1751	N	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl propionate	1752	N	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl butanoate	1753	N	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl isobutyrate	1754	N	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl hexanoate	1755	N	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl octanoate	1756	N	No safety concern
2-(4-Methyl-5-thiazolyl)ethyl decanoate	1757	N	No safety concern
2,5-Dimethylthiazole	1758	N	No safety concern
2-Acetyl-2-thiazoline	1759	N	No safety concern
2-Propionyl-2-thiazoline	1760	N	No safety concern
2-Hexylthiophene	1764	N	No safety concern
5-Acetyl-2,3-dihydro-1,4-thiazine	1766	N	No safety concern
Structural class III			
cis- and *trans*-5-Ethyl-4-methyl-2-(2-methylpropyl) thiazoline	1761	N	No safety concern
cis- and *trans*-5-Ethyl-4-methyl-2-(1-methylpropyl) thiazoline	1762	N	No safety concern
Pyrrolidino-[1,2e]-4H-2,4-dimethyl-1,3,5-dithiazine	1763	N	No safety concern
3-(Methylthio)-methylthiophene	1765	N	No safety concern

[a] N: new specifications prepared.

G. Aliphatic and aromatic amines and amides

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
4-Aminobutyric acid	1771	N	No safety concern
N-Gluconyl ethanolamine	1772	N	No safety concern
N-Gluconyl ethanolamine phosphate	1773	N	No safety concern
N-Lactoyl ethanolamine	1774	N	No safety concern
N-Lactoyl ethanolamine phosphate	1775	N	No safety concern
Structural class III			
N-(Heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide	1767	N	No safety concern
*N*1-(2,4-Dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide	1768	N	No safety concern
*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide	1769	N	No safety concern
*N*1-(2-Methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide	1770	N	No safety concern
N-[(Ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide	1776	N	No safety concern
N-[2-(3,4-Dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide	1777	N	No safety concern
N-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide	1779	N	No safety concern

[a] N: new specifications prepared.

H. Aliphatic alicyclic linear α,β-unsaturated di- and trienals and related alcohols, acids and esters

Flavouring agent	JECFA No.	Specifications[a]	Conclusions based on current estimated intake
Structural class I			
2,4-Hexadienyl acetate	1780	N	No safety concern
2,4-Hexadienyl propionate	1781	N	No safety concern
2,4-Hexadienyl isobutyrate	1782	N	No safety concern
2,4-Hexadienyl butyrate	1783	N	No safety concern
2,4-Heptadien-1-ol	1784	N	No safety concern
Nona-2,4,6-trienal	1785	N	No safety concern
2,4,7-Decatrienal	1786	N	No safety concern

[a] N: new specifications prepared.

Annex 3

Further information required or desired

Cyclotetraglucose and cyclotetraglucose syrup

Data are required on the identity of the bacterial strain used to produce the 6-GT/IMT enzyme preparation and evidence of its lack of pathogenicity and toxigenicity. For cyclotetraglucose syrup, information is needed on total saccharide content and test methods and the unidentified saccharide fraction.

Estragole

The evaluation of estragole was deferred to a future meeting, pending submission of data requested for the assessment of safety and specifications for use as a flavouring agent.

Steviol glycosides

The results of the ongoing toxicological and clinical studies, in particular studies addressing pharmacological effects, should be submitted by the end of 2008.

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
Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters				
1622	*trans*-4-Hexenal	76	16–20% *cis*-4-hexenal, 2–4% *cis*-3-hexen-1-ol, 1–2% hexanal	*cis*-4-Hexenal (No. 319) was evaluated by the Committee in 1998. It was concluded that *cis*-4-hexenal was not a safety concern at current levels of intake. *cis*-3-Hexen-1-ol (No. 315) was evaluated by the Committee in 1998. It was concluded that *cis*-3-hexen-1-ol was not a safety concern at current levels of intake. In a 98-day rat drinking-water study, *cis*-3-hexen-1-ol exhibited a NOEL of 120–180 mg/kg bw per day (Gaunt et al., 1969). Hexanal (No. 92) was evaluated by the Committee in 1997. It was concluded that hexanal was not a safety concern at current levels of intake.
1636	(*Z*)-4-Dodecenal	94	3–4% dodecanal	Dodecanal is expected to share the same metabolic fate as (*Z*)-4-dodecenal and the other saturated and unsaturated aliphatic alcohols, aldehydes, carboxylic acids and related esters in this group (Dawson et al., 1964; Gaillard & Derache, 1965).
1637	*cis*-9-Octadecenol	85	8–9% hexadecanol, 5–6% octadecanol	Hexadecanol and octadecanol are expected to share the same metabolic fate as *cis*-9-octadecenol and the other saturated and unsaturated aliphatic alcohols, aldehydes, carboxylic acids and related esters in this group (Dawson et al., 1964; Gaillard & Derache, 1965).

1638	*cis*-9-Octadecenyl acetate	92	2–3% hexadecyl acetate, 2–3% octadecyl acetate	Hexadecyl acetate and octadecyl acetate are anticipated to share the same metabolic fate as *cis*-9-octadecenyl acetate and the other alcohols, aldehydes, carboxylic acids and related esters in this group (Gangolli & Shilling, 1968; Longland et al., 1977; Drake et al., 1978; Heymann, 1980; Graffner-Nordberg et al., 1998; Hosokawa et al., 2001).
1641	9-Octadecenal	94	3–5% octadecenal	Octadecenal is anticipated to share the same metabolic fate as 9-octadecenal and the other alcohols, aldehydes, carboxylic acids and related esters in this group (Dawson et al., 1964; Gaillard & Derache, 1965).
1642	(*E*)-4-Nonenal	93	1–2% 2-nonen-4-ol, 5–6% 2*E*,4*E*-nonadienal	2-Nonen-4-ol and 2*E*,4*E*-nonadienal are anticipated to share the same metabolic fate as (*E*)-4-nonenal and the other alcohols, aldehydes, carboxylic acids and related esters in this group (Dawson et al., 1964; Gaillard & Derache, 1965).
Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances				
1650	*p*-α,α-trimethylbenzyl alcohol	90	9–11% *p*-isopropenyltoluene	*p*-Isopropenyltoluene (No. 1333, *p*,α-dimethylstyrene) was evaluated by the Committee in 2004. It was concluded that *p*,α-dimethylstyrene was not a safety concern at current levels of intake. In a 90-day rat study, *p*,α-dimethylstyrene exhibited NOELs of 0.63 and 0.62 mg/kg bw per day for males and females, respectively (Posternak et al., 1969).
1654	α,α-Dimethylphenethyl formate	93	5–7% α,α-dimethylphenethyl alcohol	α,α-Dimethylphenethyl alcohol is anticipated to share the same metabolic fate as the other tertiary terpenoid alcohols in this group (Williams, 1959; Parke et al., 1974; Horning et al., 1976; Ventura et al., 1985).

Simple aliphatic and aromatic sulfides and thiols				
1660	Ethane-1,1-dithiol	1	Owing to malodorous nature, available only as a 1% solution in ethanol	Ethanol (No. 41) was evaluated by the Committee in 1996. It was concluded that ethanol was not a safety concern at current levels of intake.
1670	4-Mercapto-2-pentanone	1	Owing to malodorous nature, available only as a 1% solution in acetoin	Acetoin (No. 405) was evaluated by the Committee in 1998. It was concluded that acetoin was not a safety concern at current levels of intake.
1672	Diisopentyl thiomalate	94	2–3% diisopentyl thiotartronate	Diisopentyl thiotartronate is anticipated to undergo simultaneous metabolism of sulfur and oxygenated functional groups (Gachon et al., 1988; Karim et al., 1988; Feng & Solsten, 1991; Wilson et al., 1991; Black et al., 1993). Sulfoxide formation is usually the predominant metabolic detoxication pathway.
1673	*cis*- and *trans*-Mercapto-*p*-menthan-3-one	89	8–9% piperitone, 1–2% α-terpineol	Piperitone (No. 435) was evaluated by the Committee in 1998. It was concluded that piperitone was not a safety concern at current levels of intake. α-Terpineol (No. 366) was evaluated by the Committee in 1998. It was concluded that α-terpineol was not a safety concern at current levels of intake.
1684	2,4,6-Trithiaheptane	10	Owing to malodorous nature, available only as a 10% solution in triacetin	Triacetin (No. 920) was evaluated by the Committee in 2002. It was concluded that triacetin was not a safety concern at current levels of intake.
1685	(±)-2,8-Epithio-*cis*-*p*-menthane	93	5–6% d-limonene	d-Limonene (No. 1324) was evaluated by the Committee in 2004. It was concluded that d-limonene was not a safety concern at current levels of intake.
1687	Mixture of 3,6-diethyl-1,2,4,5-tetrathiane and 3,5-diethyl-1,2,4-trithiolane	1	Owing to malodorous nature, available only as a 1% solution in vegetable oil	Vegetable oil is a common component of traditional foods.

1692	(±)-3-(Methylthio)heptanal	92	5–7% 2-(*E*)-heptenal	2-(*E*)-Heptenal (No. 1360, *trans*-2-heptenal) was evaluated by the Committee in 2004. It was concluded that 2-(*E*)-heptenal was not a safety concern at current levels of intake.
1693	Ethyl methyl disulfide	80	8–10% dimethyl disulfide, 7–8% diethyl disulfide	Dimethyl disulfide (No. 564) was evaluated by the Committee in 1999. It was concluded that dimethyl disulfide was not a safety concern at current levels of intake. Diethyl disulfide (No. 1699) was evaluated by the Committee at the present meeting. It was concluded that diethyl disulfide was not a safety concern at current levels of intake. Diethyl disulfide is anticipated to undergo reduction to ethylthiol with subsequent methylation. Ethyl methyl sulfide is oxidized and eliminated in the urine (Snow, 1957).
1695	Ethyl propyl trisulfide	50	20–30% diethyl trisulfide, 20–30% dipropyl trisulfide	Diethyl trisulfide (No. 1701) was evaluated by the Committee at the present meeting. It was concluded that diethyl trisulfide was not a safety concern at current levels of intake. Diethyl trisulfide is predicted to be converted rapidly to the corresponding disulfide with subsequent reduction to thiol (Moutiez et al., 1994), which is then metabolized via the various pathways for simple thiols. Dipropyl trisulfide (No. 585) was evaluated by the Committee in 1999. It was concluded that dipropyl trisulfide was not a safety concern at current levels of intake.
1696	Methyl isopentyl disulfide	92	3–5% crotonic acid	Crotonic acid (No. 1371, (*E*)-2-butenoic acid) was evaluated by the Committee in 2004. It was concluded that (*E*)-2-butenoic acid was not a safety concern at current levels of intake.

1698	Butyl ethyl disulfide	90	2–3% diethyl disulfide, 5–6% dibutyl disulfide	Diethyl disulfide (No. 1699) was evaluated by the Committee at the present meeting. It was concluded that diethyl disulfide was not a safety concern at current levels of intake. Diethyl disulfide is anticipated to undergo reduction to ethylthiol with subsequent methylation. Ethyl methyl sulfide is oxidized and eliminated in the urine (Snow, 1957). Dibutyl disulfide is anticipated to undergo reduction to the corresponding thiol, which will be methylated. Butyl methyl sulfide will be oxidized and eliminated in the urine (Snow, 1957).
1700	Allyl propyl disulfide	93	1–2% allyl propyl sulfide, 1–2% dipropyl sulfide	Allyl propyl sulfide and dipropyl sulfide are anticipated to undergo rapid oxidation to form the corresponding sulfoxides and potentially sulfones, which are eliminated in the urine (Damani, 1987).
1709	bis-(1-Mercaptopropyl)sulfide	56	36% 3,5-diethyl-1,2, 4-trithiolane, approximately 5% dipropyl trisulfide	3,5-Diethyl-1,2,4-trithiolane (No. 1686) was evaluated by the Committee at the present meeting. It was concluded that 3,5-diethyl-1,2,4-trithiolane was not a safety concern at current levels of intake. 3,5-Diethyl-1,2,4-trithiolane is anticipated to undergo oxidation and subsequent elimination in the urine or reduction to the free dithiol (Nelson & Cox, 2000). Dipropyl trisulfide (No. 585) was evaluated by the Committee in 1999. It was concluded that dipropyl trisulfide was not a safety concern at current levels of intake.
1717	1-Hydroxy-2-butanone	90	5–10% acetoin	Acetoin (No. 405) was evaluated by the Committee in 1998. It was concluded that acetoin was not a safety concern at current levels of intake.

Aliphatic and aromatic amines and amides				
1774	*N*-Lactoyl ethanolamine	90	6–8% 2-aminoethanol lactate	2-Aminoethanol lactate is anticipated to undergo hydrolysis to form ethanolamine and lactic acid (Schmid et al., 1985). Lactic acid (No. 930) was evaluated by the Committee in 2001. It was concluded that lactic acid was of no safety concern at current levels of intake. 2-Aminoethanol is anticipated to undergo conjugation with glucuronic acid via the alcohol moiety, or, as a primary aliphatic amine with an accessible α-substituted carbon atom, it may be *N*-oxidized to nitroso groups and subsequently oximes by cytochrome P450 enzymes (Uehleke, 1973).
1775	*N*-Lactoyl ethanolamine phosphate	90	6–10% ammonium formate	Ammonium formate is anticipated to hydrolyse to form ammonia and formic acid. Formic acid (No. 79) was evaluated by the Committee in 1997. It was concluded that formic acid was not a safety concern at current levels of intake. Ammonia found in the intestinal lumen by either ingestion or endogenous production is rapidly absorbed into the portal vein and converted to urea by the liver via the Krebs-Henseleit urea cycle (Furst et al., 1969; Pitts, 1971; Mathews & van Holde, 1990; Nelson & Cox, 2000).

References for Annex 4

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