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Flavouring Group Evaluation 15, Revision 1 (FGE.15Rev1)

Aryl-substituted saturated and unsaturated primary alcohol/aldehyde/acid/ester derivatives from chemical group 22

(Commission Regulation (EC) No 1565/2000 of 18 July 2000)

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission

(Question No EFSA-Q-2003-158B)

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PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Scientific Panel is asked to evaluate nine flavouring substances in the Flavouring Group Evaluation 15, Revision 1 (FGE.15Rev1), using the procedure as referred to in the Commission Regulation (EC) No 1565/2000. These nine flavouring substances belong to chemical group 22, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation (FGE) deals with nine aryl-substituted saturated and unsaturated primary alcohol, aldehyde, carboxylic acid and ester derivatives.

Four of the nine flavouring substances can exist as stereoisomers. In each of these cases the stereoisomeric composition has not been specified.

The nine flavouring substances are classified into structural class I.

Six of the nine flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily Intakes (MSDIs) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the nine flavouring substances in this group have intakes in Europe from 0.01 to 0.37 microgram/capita/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day) substances.

The available genotoxicity data are not sufficient to evaluate the genotoxicity adequately. However, the limited data available do not preclude evaluating the nine candidate substances, using the Procedure.

All nine candidate substances in this flavouring group may be expected to be metabolised to innocuous products.

It is noted, that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation, using the Procedure.

It was considered that on the basis of the default MSDI approach these nine flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3900 microgram/person/day for the nine flavouring substances, which are all from structural class I. Thus, the intakes were all above the threshold of concern for structural class I of 1800

microgram/person/day, except for one flavouring substance [FL-no: 05.156]. This substance is also expected to be metabolised to innocuous products.

Thus, for eight of the nine flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these eight substances [FL-no: 02.173, 08.088, 08.089, 09.364 09.690, 09.735, 09.836 and 09.837] more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for five flavouring substances. Information on stereoisomerism is missing for four of the substances [FL-no: 08.088, 08.089, 09364 and 09.735] and an identity test is missing for one substance [FL-no: 09.364]. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 08.088, 08.089, 09.364 and 09.735], pending further information. The remaining five substances [FL-no: 02.173, 05.156, 09.690, 09.836 and 09.837] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

Keywords

Flavourings, alcohols, aldehydes, acids, esters, aryl-substituted, saturated, unsaturated, safety.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2006/252/EC (EC, 2006). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000), which is broadly based on the opinion of the Scientific Committee on Food (SCF, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The Flavouring Group Evaluation (FGE) is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 (EC, 2002b) and to take into account additional information that has been made available since the previous opinion on this FGE.

The revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the positive list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996).

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.15	28 June 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1182_en.html	8
FGE.15rev1	28 November 2007	http://www.efsa.europa.eu/EFSA/ScientificPanels/AFC/efsa_locale-1178620753812_Opinions425.htm	9

The present revision of FGE.15, FGE.15Rev1, includes the assessment of one additional candidate substance [FL-no: 09.364]. No additional toxicity or metabolism data were available for this substance. Otherwise no additional information was made available since FGE.15 was published.

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 15, Revision 1

1.1. Description

The present Flavouring Group Evaluation, Revision 1 (FGE.15Rev1), using the procedure as referred to in the Commission Regulation (EC) No 1565/2000 (The Procedure –shown in schematic form in Annex I), deals with nine flavouring substances (candidate substances) from chemical group 22, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000).

The nine candidate substances under consideration, with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1. This group of candidate substances includes saturated and unsaturated phenylpropane derivatives: one alcohol [FL-no: 02.173], one aldehyde [FL-no: 05.156], two carboxylic acids [FL-no: 08.088 and 08.089] and five related esters [FL-no: 09.364, 09.690, 09.735, 09.836 and 09.837]. The four non-esterified compounds are substituted with one or two hydroxy- and/or methoxy-groups on the phenyl ring.

The nine candidate substances are structurally related to 29 flavouring substances (supporting substances) evaluated at the 55th JECFA meeting (JECFA, 2001a). These substances, with the respective structural formulas, FEMA, CoE and CAS register numbers, evaluation status by Scientific Committee on Food (SCF), the JECFA and CoE, and the European Maximised Survey-derived Daily Intake (MSDI) values are listed in Table 3.

The supporting substances are also based on the phenylpropane structure, except for one phenylpentane derivative [FL-no: 02.051]. The group includes saturated and unsaturated derivatives: two alcohols, three aldehydes, two acids, and twenty-two related esters. Of the two acids, one is saturated in the propyl chain (phenylpropionic acid) and one unsaturated (cinnamic acid = 3-phenyl-2-propenoic acid). Six of the esters are derived from phenylpropanol and the other sixteen esters are derived from the unsaturated cinnamic acid. None of the supporting substances are hydroxylated or methoxylated in the phenyl ring.

The hydrolysis products of the candidate esters are listed in Table 2b.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variation of their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce.

Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

One candidate substance, ethyl 2-phenylpropionate [FL-no: 09.364], possess a chiral centre. The substance has been presented without specification of the stereoisomeric composition.

Due to the presence and the position of double bonds, three of the nine candidate substances can exist as geometrical isomers, 4-hydroxy-3,5-dimethoxycinnamic acid [FL-no: 08.088], 4-hydroxy-3-methoxycinnamic acid [FL-no: 08.089] and pentyl cinnamate [FL-no: 09.735]. In each of these cases, no indication of the preponderance of either of the possible isomers in the commercial flavouring material has been given (see Table 1). For the three flavouring substances Industry has informed that they exist as a "mixture of isomers". However, the Panel does not consider this information sufficient and requests data on the actual ratios.

1.3. Natural Occurrence in Food

Six of the nine candidate substances have been reported to occur in coffee, beer, various types of alcoholic beverages, asparagus, black currants and/or soybean. Quantitative data on the natural occurrence in food have been reported for three of these substances.

These reports include among others:

- 3-(4-Hydroxy-3-methoxyphenyl)propanal [FL-no: 05.156]: Up to 0.8 mg/kg in coffee.
- 4-Hydroxy-3,5-dimethoxycinnamic acid [FL-no: 08.088]: Up to 0.3 mg/kg in beer, up to 13 mg/kg in soybean, and up to 10 mg/kg in wine.
- 4-Hydroxy-3-methoxycinnamic acid [FL-no: 08.089]: Up to 2 mg/kg in beer, 35 mg/kg in asparagus (raw), up to 19 mg/kg in black currants, up to 0.4 mg/kg in spirits, up to 0.1 mg/kg in soybean, up to 12 mg/kg in wine.

Three of the candidate substances [FL-no: 02.173, 09.836 and 09.837] have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

2. Specifications

Purity criteria for the nine candidate substances have been provided by the flavouring industry (EFFA, 2003m). Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria is for the nine candidate substances, except that an identity test is missing for one substance [FL-no: 09.364], and stereoisomeric information is needed for four candidate substances [FL-no: 08.088, 08.089, 09.364 and 09.735] (see Section 1.2 and Table 1).

3. Intake data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production

figure only represents 60 % of the use in food due to underreporting and that 10% of the total EU population are consumers (SCF, 1999).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The Maximised Survey-derived Daily Intake (MSDI (SCF, 1999)) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population[1] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

[1] EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

In the present Flavouring Groupo Evaluation 15, Revision 1 (FGE.15Rev1) the total annual volume of production of the nine candidate substances from use as flavouring substances in Europe has been reported to be approximately 5.7 kg (EFFA, 2003n; EFFA, 2004ab). For 24 of the 29 supporting substances the total annual volume of production in Europe is approximately 22000 kg (methyl cinnamate [FL-no: 09.740] accounts for 19000 kg) (JECFA, 2001b). The annual volumes of production in Europe for five of the supporting substances [FL-no: 02.051, 05.094, 09.071, 09.084 and 09.746] were not reported.

On the basis of the annual volumes of production reported for the nine candidate substances, the daily *per capita* intake for each of these flavourings has been estimated (Table 2a).

Approximately 50 % of the total annual volume of production for the candidate substances is accounted for by 3-phenylpropyl benzoate [FL-no: 09.836]. The estimated daily *per capita* intake of this candidate substance from use as flavouring substance is 0.37 microgram. The daily *per capita* intakes for each of the remaining substances is less than 0.12 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume specified amounts of flavourable foods and beverages per day (see Table II.2.1, Annex II).

For the nine candidate substances information on food categories and normal and maximum use levels[2,3,4] were submitted by the Flavour Industry (EFFA, 2003m; EFFA, 2004ab; EFFA, 2007a).

The nine candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

According to the Flavour Industry the normal use levels for the nine candidate substances are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2003m; EFFA, 2004ab; EFFA, 2007a).

The mTAMDI values for the nine candidate substances from structural class I (see Section 5) range from 1600 to 3900 microgram/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

[2] ”Normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i).
[3] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).
[4] The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 1	Dairy products, excluding products of category 2	All 9
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All 9
Category 3	Edible ices, including sherbet and sorbet	All 9
Category 4.1	Processed fruits	All 9
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 5	Confectionery	All 9
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 9
Category 7	Bakery wares	All 9
Category 8	Meat and meat products, including poultry and game	All 9
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All 9
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All 9
Category 13	Foodstuffs intended for particular nutritional uses.	All 9
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 9
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 9
Category 15	Ready-to-eat savouries	All 9
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 9

4. Absorption, Distribution, Metabolism and Elimination

A more detailed description of the metabolism is given in Annex III.

Information on kinetics is very limited on candidate substances and conclusions are therefore mainly based on supporting substances.

The supporting substance cinnamic acid, its methyl ester and other structurally related derivatives as well as the saturated analogue 3-phenylpropanoic acid and its derivatives, have been shown to be rapidly absorbed from the gut.

Esters of cinnamic acid and structurally related aromatic esters have been shown to be hydrolysed to the component carboxylic acid and alcohol. It is therefore anticipated that the candidate ester pentyl cinnamate [FL-no: 09.735] is hydrolysed to cinnamic acid and pentyl alcohol, and the candidate esters 3-phenylpropyl benzoate and 3-phenylpropyl 3-phenylpropionate [FL-no 09.836

and 09.837] would be hydrolysed to benzoic acid and 3-phenylpropanoic acid, respectively, and in both cases to the aromatic alcohol 3-phenylpropanol. It is anticipated that the candidate ester 3-phenylpropyl butyrate [FL-no: 09.690] would be hydrolysed to butanoic acid and 3-phenylpropanol and the candidate ester ethyl 2-phenylpropionate [FL-no: 09.364] to ethanol and 2-phenylpropionic acid.

3-Phenylpropanol formed by the hydrolysis of candidate esters [FL-no: 09.690, 09.836 and 09.837] may subsequently be oxidised to 3-phenylpropanoic acid. The candidate aromatic primary alcohol [FL-no: 02.173] and aromatic aldehyde [FL-no: 05.156] are similarly expected to be oxidised to 3-(4-methoxyphenyl)propanoic acid and 3-(4-hydroxy-3-methoxyphenyl)propanoic acid, respectively.

Aromatic carboxylic acids, such as cinnamic acid and its saturated analogue 3-phenylpropanoic acid, are expected to be primarily converted to CoA esters and either to form conjugates with glycine or to undergo further beta-oxidation leading to the formation of benzoyl CoA. *Meta*- and *para*-substituents, in relation to the three carbon side chain, which are present in [FL-no: 02.173 and 05.156] have no significant effect on the metabolism via beta-oxidation. Benzoyl CoA that is formed from these reactions may be conjugated with glycine and excreted as hippuric acid, or may be hydrolysed to yield free benzoic acid which is then excreted. The same reactions are predicted to occur for the substituted benzoic acid CoA esters.

Conclusion

It is concluded that the nine candidate substances in this flavouring group may be expected to be metabolised to innocuous products.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where, the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the nine candidate substances from chemical group 22 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the nine substances are summarised in Table 2A.

Step 1

All nine candidate substances are classified into structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

At the estimated levels of intake all nine candidate substances are expected to be metabolised to innocuous products. Accordingly, all nine flavouring substances in the present Flavouring Group Evaluation proceed via the A-side of the Procedure scheme (Annex I).

Step A3

The estimated levels of the European daily *per capita* intake (MSDI) for the nine candidate substances, which have all been classified into structural class I, are for each less than 0.4 microgram (Table 2a). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

Based on results of the safety evaluation sequence, the nine candidate substances are not expected to be of safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

6. Comparison of the Intake Estimations based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for the nine candidate substances in structural class I based on the mTAMDI range from 1600 to 3900 microgram/person/day. With the exception of 3-(4-hydroxy-3-methoxyphenyl)propanal [FL-no: 05.156] the mTAMDI values are above the threshold of concern for structural class I substances of 1800 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

For eight candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.173	3-(4-Methoxyphenyl)propan-1-ol	0.061	3900	Class I	1800
05.156	3-(4-Hydroxy-3-methoxyphenyl)propanal	0.12	1600	Class I	1800
08.088	4-Hydroxy-3,5-dimethoxycinnamic acid	0.012	3200	Class I	1800
08.089	4-Hydroxy-3-methoxycinnamic acid	0.097	3200	Class I	1800
09.364	Ethyl 2-phenylpropionate	0.0024	3900	Class I	1800
09.690	3-Phenylpropyl butyrate	0.012	3900	Class I	1800
09.735	Pentyl cinnamate	0.012	3900	Class I	1800
09.836	3-Phenylpropyl benzoate	0.37	3900	Class I	1800
09.837	3-Phenylpropyl 3-phenylpropionate	0.012	3900	Class I	1800

7. Considerations of Combined Intakes From Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this Flavouring Group Evaluation (FGE) may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The estimated combined daily *per capita* intake is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003n; EFFA, 2003ab), the combined estimated daily *per capita* intake as flavourings of the nine candidate flavouring substances assigned to structural class I is 0.7 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day.

The nine candidate substances are structurally related to 29 supporting substances evaluated by JEFCA at its 55th meeting (JECFA, 2001b). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 24 of the 29 supporting substances. Production volumes in Europe were not reported for five of the supporting substances [FL-no: 02.051, 05.094, 09.071, 09.084 and 09.746].

The total combined intake of the nine candidate substances and 24 supporting substances from structural class I, is approximately 2700 microgram/*capita*/day, which exceeds the threshold of concern for a compound belonging to structural class I of 1800 microgram/*capita*/day.

The estimated total combined intake, based on the MSDI approach, of 2700 microgram/*capita*/day, equivalent to 45 microgram/kg body weight (bw)/day is approximately 10,000 times lower than the NOAEL of 500 mg/kg bw/day for the supporting substances linalyl- and benzyl cinnamate.

Further, at the level of exposure resulting from the use as flavourings, all the candidate and supporting substances are expected to be efficiently metabolised and would not be expected to saturate the metabolic pathways.

Therefore, it can be concluded that the total combined intake of the nine candiate substances and the 24 supporting substances does not pose a safety concern.

8. Toxicity

8.1. Acute Toxicity

Data are available for one candidate substance and for eighteen structurally related supporting substances evaluated by JECFA (JECFA, 2001b). The oral LD_{50} values in rats and mice are in the range of 2000 up to more than 5000 mg/kg bw.

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data on subacute oral toxicity are available for one candidate substance [FL-no: 08.089]. Data on subchronic oral toxicity are available for four supporting substances [FL-no: 09.730, 09.736, 09.738 and 09.740]. There are no data available on chronic toxicity and carcinogenicity for either candidate or supporting substances.

No treatment-related adverse effects could be observed in any of the available studies at the applied dose-levels (i.e. single dose-level of 2000 mg/kg bw/day in the subacute study on the one candidate substance [FL-no: 08.089] or dose-levels ranging up to 500 mg/kg bw/day in the subchronic studies on supporting substances [FL-no: 09.730, 09.736, 09.738 and 09.740]). In the multiple dose studies on linalyl cinnamate and benzyl cinnamate [FL-no: 09.736 and 09.738] the NOAEL was taken to be 500 mg/kg bw/day, the highest dose tested.

Data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

There are no data available for candidate substances. For supporting substances there is one developmental toxicity study available for cinnamic acid [FL-no: 08.022] in which no adverse effects were observed at any of the applied dose levels up to 50 mg/kg bw/day in rats. The study is summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Limited *in vitro* genotoxicity data are available for only two candidate [FL-no: 08.088 and 08.089] and for six supporting substances [FL-no: 05.080, 08.022, 09.730, 09.738, 09.740 and 09.744].

The mutagenicity studies available on the candidate substances 4-hydroxy-3,5-dimethoxycinnamic acid [FL-no: 08.088] and 4-hydroxy-3-methoxycinnamic acid [FL-no: 08.089] are considered to provide little useful information regarding the genotoxicity of the candidate substances.

4-Hydroxy-3-methoxycinnamic acid [FL-no: 08.089] was tested for its influence on spontaneous as well as induced sister chromatid exchange (SCE) in cultured Chinese hamster ovary (CHO) cells only in the absence of metabolic activation. The result was negative.

The five supporting substances [FL-no: 05.080, 08.022, 09.730, 09.738 and 09.744] have been tested for their ability to induce mutations in various strains of *Salmonella typhimurium* (e.g. TA92, TA94, TA98, TA100, TA1535, TA1537 and TA1538), in the presence or absence of an exogenous metabolic activation system. None of the compounds was mutagenic when tested at concentrations

up to 5000 microgram/plate. Four of the substances, cinnamic acid [FL-no: 08.022], methyl cinnamate [FL-no: 09.740], ethyl cinnamate [FL-no: 09.730] and 3-phenylpropionaldehyde [FL-no: 05.080] were tested for induction of spontaneous SCEs in cultured CHO cells only in the absence of metabolic activation. For all the four substances no influence on cell cycle and SCE was observed. Ethyl cinnamate [FL-no: 09.730] in a study carried out in the absence of S9 activation did not induce chromosomal aberrations in Chinese hamster fibroblasts.

There are no *in vivo* genotoxicity data available for the candidate and supporting substances in the present flavouring group evaluation.

Conclusion on genotoxicity:

Overall, the data available are not sufficient to evaluate the genotoxicity adequately and no *in vivo* genotoxicity data are available for the candidate or for the supporting substances, but the various studies carried out with supporting substances give no indication of a mutagenic activity in bacterial cells or of a direct clastogenic effect on mammalian cells. The limited genotoxicity data available do not preclude evaluating the nine candidate substances, using the Procedure.

Data are summarised in Annex IV, Table IV.4.

9. Conclusions

The nine candidate substances are aryl-substituted saturated and unsaturated primary alcohol, aldehyde, acid and ester derivatives belonging to chemical group 22.

One candidate substance, ethyl 2-phenylpropionate [FL-no: 09.364], possess a chiral centre. The substance has been presented without specification of the stereoisomeric composition.

Due to the presence and the position of the double bonds, three of the nine flavouring substances can exist as geometrical isomers [FL-no: 08.088, 08.089 and 09.735]. In all three cases, no indication has been given that one of the possible isomers has preponderance in the commercial flavouring material.

The nine flavouring substances are classified into structural class I.

Six of the substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the nine flavouring substances in this group have intakes in Europe from 0.01 to 0.37 microgram/*capita*/day, which are below the threshold of concern value for structural class I substances of 1800 microgram/person/day.

On the basis of the reported annual production in Europe (MSDI approach) the combined intake of the nine candidate substances, all belonging to structural class I, would result in a total intake of approximately 0.7 microgram/*capita*/day. This value is lower than the threshold of concern for structural class I substances. The total combined estimated level of intake of 24 of the 29 supporting substances for which European annual production data are available and of the nine candidate substances is approximately 2700 microgram/*capita*/day, which exceeds the threshold of concern

for structural class I (1800 microgram/person/day). However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

The genotoxicity data available are not sufficient to evaluate the genotoxicity adequately. However, the limited data available do not preclude evaluation of the nine candidate substances, using the Procedure.

The nine candidate substances in this flavouring group may be expected to be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is considered that on the basis of the default MSDI approach these nine candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 1600 to 3900 microgram/person/day for the nine flavouring substances from structural class I. The intakes were above the threshold of concern for structural class I of 1800 microgram/person/day, except for one flavouring substance [FL-no: 05.156]. This substance is expected to be metabolised to innocuous products.

Thus, for eight of the nine flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substances have been assigned. Therefore, for these eight substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the nine candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for five flavouring substances. Information on stereoisomerism is missing for four of the substances [FL-no: 08.088, 08.089, 09.364 and 09.735] and an identity test is missing for one substance [FL-no: 09.364]. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 08.088, 08.089, 09.364 and 09.735], pending further information. The remaining five substances [FL-no: 02.173, 05.156, 09.690, 09.836 and 09.837] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 15, REVISION 1

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 15, Revision 1

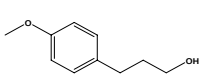
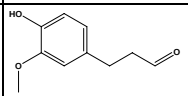
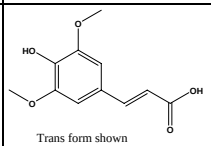
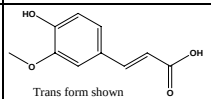
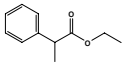
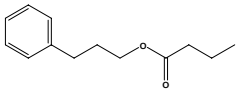
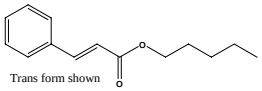
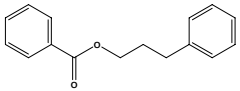
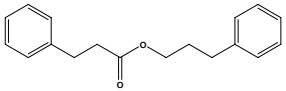
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.173	3-(4-Methoxyphenyl)propan-1-ol		5406-18-8	Solid $C_{10}H_{14}O_2$ 166.22	Practically insoluble or insoluble 1 ml in 1 ml	161 (13 hPa) 26 MS 95 %	n.a. n.a.	
05.156	3-(4-Hydroxy-3-methoxyphenyl)propanal		80638-48-8	Solid $C_{10}H_{12}O_3$ 180.20	Slightly soluble 1 ml in 1 ml	339 144 NMR 95 %	n.a. n.a.	
08.088	4-Hydroxy-3,5-dimethoxycinnamic acid 6)	 Trans form shown	530-59-6	Solid $C_{11}H_{12}O_5$ 224.21	Slightly soluble 1 ml in 1 ml	490 192 MS 95 %	n.a. n.a.	(Z)- or (E)- isomer not specified by CASrn in Register.
08.089	4-Hydroxy-3-methoxycinnamic acid 6)	 Trans form shown	10113 1135-24-6	Solid $C_{10}H_{10}O_4$ 194.19	Slightly soluble 1 ml in 1 ml	439 171 MS 98 %	n.a. n.a.	(Z)- or (E)- isomer not specified by CASrn in Register.
09.364	Ethyl 2-phenylpropionate 6)		2510-99-8	Solid $C_{11}H_{14}O_2$ 178.23	Practically insoluble or insoluble 1 ml in 1 ml	230 69 95 %	1.490-1.496 1.019-1.025	ID 7) (R) or (S) enantiomer not specified by CASrn in Register.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 15, Revision 1

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.690	3-Phenylpropyl butyrate		7402-29-2	Solid $C_{13}H_{18}O_2$ 206.28	Practically insoluble or insoluble 1 ml in 1 ml	280 31 MS 95 %	n.a. n.a.	CASrn to be changed to 7402-29-1.
09.735	Pentyl cinnamate 6)		328 3487-99-8	Liquid $C_{14}H_{18}O_2$ 218.29	Practically insoluble or insoluble 1 ml in 1 ml	312 MS 95 %	1.538-1.544 0.992-0.998	(Z)- or (E)- isomer not specified by CASrn in Register.
09.836	3-Phenylpropyl benzoate		60045-26-3	Solid $C_{16}H_{16}O_2$ 240.30	Practically insoluble or insoluble 1 ml in 1 ml	338 92 MS 95 %	n.a. n.a.	
09.837	3-Phenylpropyl 3-phenylpropionate		60045-27-4	Solid $C_{18}H_{20}O_2$ 268.35	Practically insoluble or insoluble 1 ml in 1 ml	148 (1 hPa) 114 MS 95 %	n.a. n.a.	

1) Solubility in water, if not otherwise stated.

2) Solubility in 95% ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

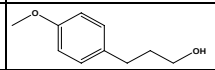
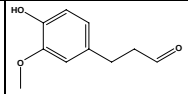
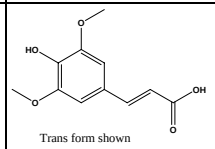
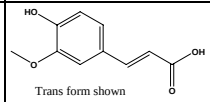
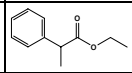
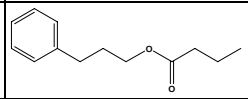
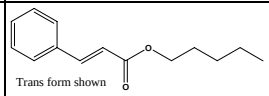
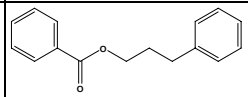
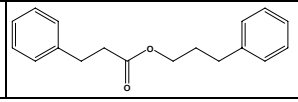
6) Stereoisomeric composition not specified.

7) ID: Missing identification test.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

Flavouring Group Evaluation 15, Revision 1 (FGE.15Rev1). Aryl-substituted saturated and unsaturated primary alcohol/aldehyde/acid/ester derivatives from chemical group 22

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.173	3-(4-Methoxyphenyl)propan-1-ol		0.061	Class I A3: Intake below threshold	4)	6)	
05.156	3-(4-Hydroxy-3-methoxyphenyl)propanal		0.12	Class I A3: Intake below threshold	4)	6)	
08.088	4-Hydroxy-3,5-dimethoxycinnamic acid	 Trans form shown	0.012	Class I A3: Intake below threshold	4)	7)	
08.089	4-Hydroxy-3-methoxycinnamic acid	 Trans form shown	0.097	Class I A3: Intake below threshold	4)	7)	
09.364	Ethyl 2-phenylpropionate		0.0024	Class I A3: Intake below threshold	4)	7)	
09.690	3-Phenylpropyl butyrate		0.012	Class I A3: Intake below threshold	4)	6)	
09.735	Pentyl cinnamate	 Trans form shown	0.012	Class I A3: Intake below threshold	4)	7)	
09.836	3-Phenylpropyl benzoate		0.37	Class I A3: Intake below threshold	4)	6)	
09.837	3-Phenylpropyl 3-phenylpropionate		0.012	Class I A3: Intake below threshold	4)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

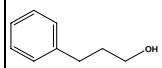
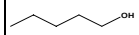
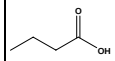
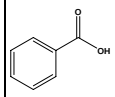
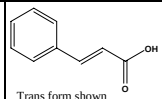
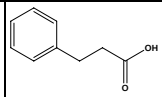
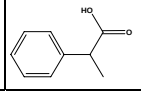
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.031	3-Phenylpropan-1-ol 636		No safety concern a) Category B b)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 c) No safety concern e)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
08.005	Butyric acid 87		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.021	Benzoic acid 850		No safety concern f) Deleted b)	Class I A3: Intake below threshold	Substances for which CoE Committee of Experts had no information as to real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992) .
08.022	Cinnamic acid 657	 Trans form shown	No safety concern a) Category A b)	Class I A3: Intake below threshold	
08.032	3-Phenylpropionic acid 646		No safety concern a) Category B b)	Class I A3: Intake below threshold	
08.108	2-Phenylpropionic acid		FGE.14Rev1	Class I A3: Intake below threshold	

1) Category 1: Considered safe in use. Category 2: Temporarily considered safe in use. Category 3: Insufficient data to provide assurance of safety in use. Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

4) Threshold of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (JECFA, 2001a).

b) (CoE, 1992).

c) (SCF, 1995).

d) (JECFA, 1999b).

e) (JECFA, 1997a).

f) (JECFA, 2002c).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

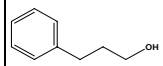
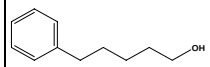
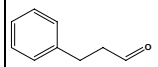
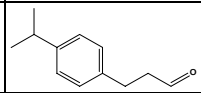
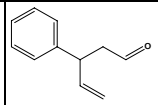
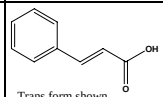
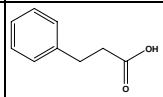
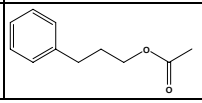
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.031	3-Phenylpropan-1-ol		2885 80 122-97-4	636 JECFA specification (JECFA, 2000d)	51	No safety concern a) Category B b)	
02.051	5-Phenylpentan-1-ol		3618 674 10521-91-2	675 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
05.080	3-Phenylpropanal		2887 2013 104-53-0	645 JECFA specification (JECFA, 2001c)	16	No safety concern a) Category B b)	
05.094	3-(4-Isopropylphenyl)propionaldehyde		2957 2261 7775-00-0	680 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
05.103	3-Phenylpent-4-enal		3318 10378 939-21-9	679 JECFA specification (JECFA, 2000d)	0.73	No safety concern a)	
08.022	Cinnamic acid	 Trans form shown	2288 22 621-82-9	657 JECFA specification (JECFA, 2000d)	28	No safety concern a) Category A b)	
08.032	3-Phenylpropionic acid		2889 32 501-52-0	646 JECFA specification (JECFA, 2000d)	20	No safety concern a) Category B b)	
09.032	3-Phenylpropyl acetate		2890 222 122-72-5	638 JECFA specification (JECFA, 2000d)	35	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

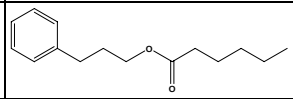
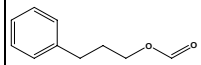
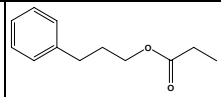
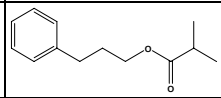
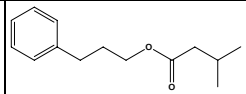
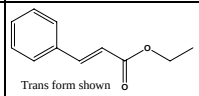
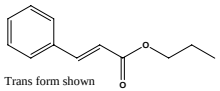
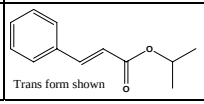
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.071	3-Phenylpropyl hexanoate		2896 321 6281-40-9	642 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
09.084	3-Phenylpropyl formate		2895 351 104-64-3	637 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
09.138	3-Phenylpropyl propionate		2897 419 122-74-7	639 JECFA specification (JECFA, 2000d)	0.12	No safety concern a) Category B b)	
09.428	3-Phenylpropyl isobutyrate		2893 303 103-58-2	640 JECFA specification (JECFA, 2000d)	3.7	No safety concern a) Category B b)	
09.467	3-Phenylpropyl isovalerate		2899 462 5452-07-3	641 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category B b)	
09.730	Ethyl cinnamate	 Trans form shown	2430 323 103-36-6	659 JECFA specification (JECFA, 2000d)	89	No safety concern a) Category B b)	
09.731	Propyl cinnamate	 Trans form shown	2938 324 7778-83-8	660 JECFA specification (JECFA, 2001c)	0.32	No safety concern a) Category B b)	
09.732	Isopropyl cinnamate	 Trans form shown	2939 325 7780-06-5	661 JECFA specification (JECFA, 2000d)	16	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

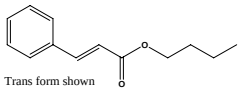
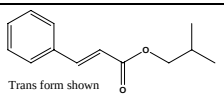
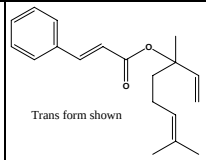
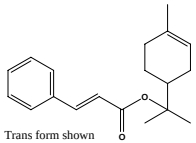
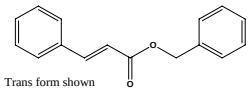
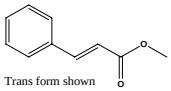
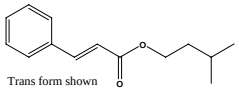
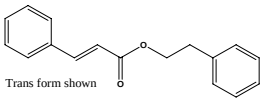
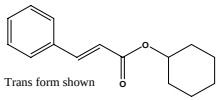
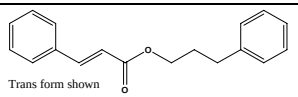
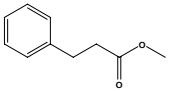
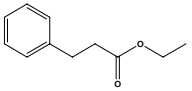
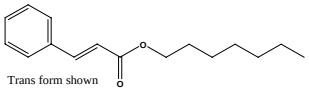
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.733	Butyl cinnamate	 Trans form shown	2192 326 538-65-8	663 JECFA specification (JECFA, 2001c)	0.37	No safety concern a) Category B b)	
09.734	Isobutyl cinnamate	 Trans form shown	2193 327 122-67-8	664 JECFA specification (JECFA, 2000d)	1.2	No safety concern a) Category B b)	
09.736	Linalyl cinnamate	 Trans form shown	2641 329 78-37-5	668 JECFA specification (JECFA, 2003b)	6.0	No safety concern a) Category A b)	
09.737	Terpinyl cinnamate	 Trans form shown	3051 330 10024-56-3	669 JECFA specification (JECFA, 2003b)	0.012	No safety concern a) Category B b)	
09.738	Benzyl cinnamate	 Trans form shown	2142 331 103-41-3	670 JECFA specification (JECFA, 2000d)	38	No safety concern a) Category A b)	
09.740	Methyl cinnamate	 Trans form shown	2698 333 103-26-4	658 JECFA specification (JECFA, 2000d)	2400	No safety concern a) Category A b)	
09.742	Isopentyl cinnamate	 Trans form shown	2063 335 7779-65-9	665 JECFA specification (JECFA, 2000d)	6.9	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.743	Phenethyl cinnamate		2863 336 103-53-7	671 JECFA specification (JECFA, 2001c)	4.9	No safety concern a) Category B b)	
09.744	Cyclohexyl cinnamate		2352 337 7779-17-1	667 JECFA specification (JECFA, 2000d)	0.37	No safety concern a) Category B b)	
09.745	3-Phenylpropyl cinnamate		2894 338 122-68-9	672 JECFA specification (JECFA, 2001c)	0.49	No safety concern a) Category B b)	
09.746	Methyl 3-phenylpropionate		2741 427 103-25-3	643 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
09.747	Ethyl 3-phenylpropionate		2455 429 2021-28-5	644 JECFA specification (JECFA, 2000d)	1.2	No safety concern a) Category B b)	
09.782	Heptyl cinnamate		2551 2104 10032-08-3	666 JECFA specification (JECFA, 2001c)	1.5	No safety concern a) Category B b)	

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = μg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2001a).

b) (CoE, 1992).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[5] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[6] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

[5] *"Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).*

[6] *"Endogenous substances": 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 (JECFA, 1997a).*

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

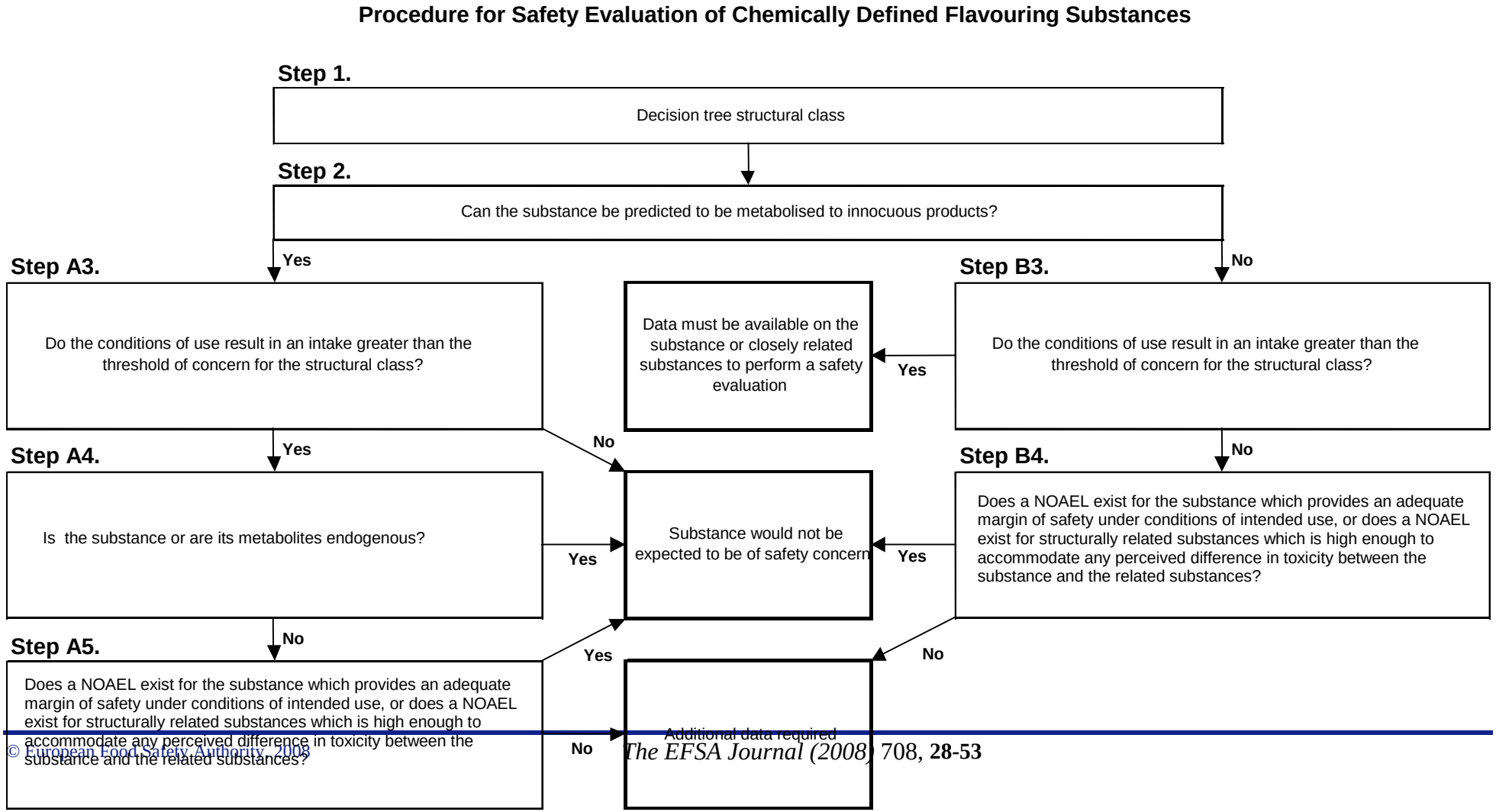


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1. Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry for the nine candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.15 (EFFA, 2003m)

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
02.173	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
05.156	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
08.088	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25
08.089	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25
09.364	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.690	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.735	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.836	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.837	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25

II.2. mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The present mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No. 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01	Dairy products, excluding products of category 02.0	Food		
02	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05	Confectionery			Exception a
06	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07	Bakery wares	Food		
08	Meat and meat products, including poultry and game	Food		
09	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10	Eggs and egg products	Food		
11	Sweeteners, including honey			Exception a

12	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15	Ready-to-eat savouries			Exception b
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for each of the nine flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003m; EFFA, 2004ab; EFFA, 2007a). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.173	3-(4-Methoxyphenyl)propan-1-ol	3900	Class I	1800
05.156	3-(4-Hydroxy-3-methoxyphenyl)propanal	1600	Class I	1800
08.088	4-Hydroxy-3,5-dimethoxycinnamic acid	3200	Class I	1800
08.089	4-Hydroxy-3-methoxycinnamic acid	3200	Class I	1800
09.364	Ethyl 2-phenylpropionate	3900	Class I	1800
09.690	3-Phenylpropyl butyrate	3900	Class I	1800
09.735	Pentyl cinnamate	3900	Class I	1800
09.836	3-Phenylpropyl benzoate	3900	Class I	1800
09.837	3-Phenylpropyl 3-phenylpropionate	3900	Class I	1800

ANNEX III: METABOLISM

III.1. General Information

The flavouring group consists of nine substances (candidate substances) from chemical group 22, "Aryl-substituted primary alcohol/aldehyde/acid/ester/acetal derivatives, including unsaturated ones". Two of the candidate substances are hydroxy-methoxy-derivatives of cinnamic acid [FL-no: 08.088; and 08.089]. Five of the candidate substances are esters, of which one is a pentyl ester of cinnamic acid [FL-no: 09.735], one is an ethyl ester of 2-phenylpropionic acid [FL-no: 09.364] and three are 3-phenylpropyl esters of benzoic acid, phenylpropionic acid and butyric acid, respectively [FL-no: 09.836, 09.837 and 09.690]. One candidate substance is a methoxyphenyl derivative of propanol [FL-no: 02.173] and one is a hydroxymethoxyphenyl derivative of propanal [FL-no: 05.156]. The candidate substances are structurally related to supporting substances evaluated at the 55th meeting of the JECFA, among these are cinnamyl alcohol, cinnamic acid and related substances.

III.2. Absorption, distribution and elimination

Cinnamic acid, its corresponding methyl ester, and other cinnamoyl derivatives as well as the saturated analogue (3-phenylpropanoic acid) and its derivatives have all been shown to be rapidly absorbed from the gut, distributed and subsequently excreted as polar metabolites (conjugates and/or free benzoic acid and cinnamic acid), primarily in the urine and, to a minor extent, in the faeces. It is anticipated that the candidate substances including the 2-phenylpropionate [FL-no: 09.364] would behave similarily since they are structurally related to the supporting chemicals.

Studies on cinnamic acid or esters of cinnamic acid

In groups of male F344 rats, 79% of an oral dose of (3-^{14}C-d_5)-cinnamic acid is excreted in the urine within 24 hours. Excretion in the faeces accounted for only minor amounts of the administered acid (0.9%). Greater than 90% of the administered dose was recovered in the urine and faeces within 72 hours. Administration of the same dose of the parent acid to CD1 mice by intraperitoneal injection resulted in a similar pattern of excretion in the urine and faeces at 24 hours (93%) and 72 hours (>93%) (Nutley, 1990).

Female Birmingham Wistar rats were given a single oral dose of 20 mg cinnamic acid or methyl cinnamate per animal. The rats weighed 240 - 400 g. The rats were killed at intervals after dosing, from 5 minutes to 4 hours, and the gut was removed and dissected into four sections; stomach, small intestine, caecum and large intestine. Amounts of cinnamic acid and methyl cinnamate were determined in the sections. At no time was more than 5% of the dose detected in the lower part of the gut. When the ester was administered, cinnamic acid was recovered as 40% of the total recovery in the large intestine, the total recovery was less than 5% of the administered dose. In another experiment in the study Doe rabbits (White New Zealand strain) were dosed with 1 mmole (162 mg) methyl cinnamate/kg body weight after which blood samples were taken from the ear vein. Female rats were similarly dosed with 1.5 mmole (243 mg) methyl cinnamate/kg, and blood

samples were taken from tail vein, portal vein and heart. No ester was detected in the peripheral blood of dosed rabbits or rats, and only traces were detected in portal and heart blood samples taken from dosed rats. The results indicate that hydrolysis of methyl cinnamate occurs mainly before or during intestinal absorption (Fahelbum & James, 1977)[7].

Studies on candidate substance 4-hydroxy-3-methoxycinnamic acid [FL-no: 08.089](ferulic acid)

Urinary recovery of unlabelled ferulic acid (4-hydroxy-3-methoxycinnamic acid) [FL-no: 08.089] was studied. Ferulic acid was administered to five male Wistar rats by gavage at a concentration of 50 mg/ml in DMSO or intravenously at doses of 50 mg/kg body weight (bw). After oral administration, 5.4 ± 4.1% of the administered dose of ferulic acid [FL-no: 08.089] was detected as unchanged compound. Similar recovery (5.1 ± 3.6%) of the glucuronide conjugate was obtained. Other metabolites and routes of excretion were not studied. After intravenous administration similar results were obtained with recoveries of 8.1 ± 5.6% of unchanged parent material and 3.4 ± 2.4% of the glucuronide conjugate. There was essentially no difference in the total recovery of the administered dose between the oral (10.5 ± 2.5%) and the intravenous (11.5 ± 5.4%) routes of dosing. This indicates that ferulic acid [FL-no: 08.089] is readily bioavailable via the oral route (Choudhury et al., 1999), The unaccounted part of the dose might be excreted via other routes or as unidentified metabolites (e.g. hippuric acid) (Scheline, 1991).

The distribution, metabolism and elimination of intraperitoneally injected (^{14}C)-ferulic acid (4-hydroxy-3-methoxy-cinnamic acid; labelled at the 2 position of the side chain) [FL-no: 08.089], was studied in female Wistar rats. Twenty-four hours after administration of a 38 mg dose (150 – 190 mg/kg bw) of the labelled compound the animals were sacrificed, and the fate of the injected substance was analysed. Radioactivity was poorly incorporated in the rat organs and tissues. Of the injected activity 19.5% was recovered in the faeces and 68% in the urine, both as free ferulic acid and as *m*-hydroxyphenylpropionic acid. The weak activity found in the respiratory CO_2 (1.95 %) indicated that only very little ferulic acid was metabolised via beta-oxidation of the side-chain (Teuchy & Van Sumere, 1971).

Bioavailability of ferulic acid [FL-no: 08.089] in one male and four female human volunteers from tomato consumption was investigated through monitoring kinetics of excretion in relation to intake. Prior to the study, volunteers fasted for 12 hours, refrained from taking antioxidant supplements for one week, and avoided special phenolic-rich foods for 48 hours. The subjects consumed a single bolus of 360 - 728 g tomatoes (equivalent to 8 g/kg bw) providing a mean of 30.1 g (21 - 44 g) ferulic acid per person. Urine samples were collected prior to and for 24 hours after tomato consumption. Excretion of ferulic acid continued progressively up to 7 - 9 hours, after which it showed no further excretion. The amount of free ferulic acid excreted over 24 hours was approximately 4 – 5% of the ingested ferulic acid. A considerable proportion was excreted as the glucuronide (conjugates not further identified) in all subjects with the relative proportions of free to conjugated forms varying between individuals. The total recovery of ferulic acid in the urine, on the basis of total free ferulic acid and feruloyl glucuronide excreted was 11 - 25% of the amount ingested (Bourne & Rice-Evans, 1998).

[7] In this paper several experiments with different dosing are reported, animals have been dosed either po in mg per animal or iv in mmoles per kg bodyweight.

Based on studies discussed above, the cinnamyl derivatives among the candidate substances and supporting substances are anticipated to be rapidly absorbed and subject to further metabolisation.

III.3 Metabolism

III.3.1 Hydrolysis of esters of cinnamic acid and other aromatic esters

In general, esters containing an aromatic ring system are expected to be hydrolysed *in vivo* to the component acid and alcohol. In contrast to aliphatic esters, which are hydrolysed by B-esterases, the most important enzymes for hydrolysis of aromatic esters are the A-esterases (arylesterases). In mammals, A-esterases occur in most tissues throughout the body but predominate in the hepatocytes (Heymann, 1980). Esters of cinnamic acid and structurally related aromatic esters have been shown to be hydrolysed to the component acid and alcohol.

Methyl cinnamate was administered orally to rats as a single dose of 50 mg per animal. After 24 hours urinary excretion showed 66% (range 62-73%) of the given dose excreted as hippuric acid (benzoylglycine, benzoic acid glycine conjugate) (4 animals), and 5% (range 0 – 23%) as glucosiduronic acid (benzoyl glucuronic acid) (6 animals). Similarily, Doe rabbits weighing 3-4 kg were dosed orally with 500 mg methyl cinnamate. Urinary excretion after 24 hours showed 56% (range 53 – 60%) of the dose excreted as hippuric acid (benzoylglycine, benzoic acid glycine conjugate) (4 animals) and 8% (range 0 – 34%) as glucosiduronic acid (benzoyl glucuronic acid) (10 animals). The metabolites formed after dosing with methyl cinnamate were identical to those excreted after dosing with cinnamic acid, and no significant quantitative difference was found, indicating that hydrolysis of the ester precedes the metabolism of the acid (Fahelbum & James, 1977)[8].

Incubation of benzyl cinnamate or benzyl acetate with simulated intestinal fluid (pancreatin) at pH 7.5 and 37°C for two hours resulted in 80% and 50% hydrolysis, respectively (Grundschober, 1977).

III.3.2 Oxidation and conjugation reactions of aromatic alcohol and aromatic aldehyde derivatives

Aromatic primary alcohols, such as candidate substance [FL-no 02.173] and the alcohols which are hydrolysis products from candidate esters [FL-no 09.690; 09.836; 09.837], may be oxidised to corresponding aldehydes. Human alcohol dehydrogenase (ADH) catalyses this oxidation (Pietruszko et al., 1973). Aldehydes, such as those resulting from the reaction mentioned above as well as candidate substance [FL-no 05.156], may be oxidised to carboxylic acids in a reaction catalysed by aldehyde dehydrogenase (ALD) (Weiner, 1980). Aromatic alcohols and aldehydes have been reported to be good substrates for horse liver ADH (Sund & Theorell, 1963) and ALD (Feldman & Weiner, 1972), respectively.

[8] In this paper several experiments with different dosing are reported, animals have been dosed either p.o. in mg per animal or i.v. in mmoles per kg bodyweight.

Fifty-two percent of a 335 mg/kg bw oral dose of cinnamyl alcohol given to four rats was recovered in 0-24 hours in the urine as the glycine conjugate of benzoic acid (hippuric acid). Ten minor metabolites cumulatively accounted for about 10% of the dose. When cinnamyl alcohol was administered to mice by intraperitoneal injection, hippuric acid was the major urinary metabolite (Nutley, 1990).

Doses of 2 and 250 mg trans [3-^{14}C] cinnamaldehyde/kg bw were given by intraperitoneal (i.p.) injection to male and female F344 rats and CD1 mice. Doses of 250 mg/kg bw were administered via oral gavage to male rats and mice only. In both species the major urinary metabolites were formed by oxidation of cinnamaldehyde to yield cinnamic acid, which was subsequently oxidised in the beta-oxidation pathway. The major urinary metabolite was hippuric acid (71-75% in mice and 73-87% in rats), accompanied by small amounts of metabolites including 3-hydroxy-3-phenylpropionic acid (0.4-4%), benzoic acid (0.4-3%) and benzoyl glucuronide (0.8-7%). The glycine conjugate of cinnamic acid was formed to a considerable extent only in the mouse (4-13%). To a small extent, glutathione conjugation of cinnamaldehyde competes with the oxidation pathway. Approximately 6-9% of either dose was excreted in 24 hours as glutathione conjugates of cinnamaldehyde. The authors concluded that the excretion pattern and metabolic profile of cinnamaldehyde in rats and mice are not systematically affected by sex, dose size or route of administration (Peters & Caldwell, 1994).

The toxicokinetic profile of cinnamaldehyde has been investigated in male F344 rats. Plasma levels of cinnamaldehyde (<0.1 microg/ml) and cinnamic acid (<1 microg/ml) were not measurable when rats, that were cannulated in the jugular vein, were administered a single oral dose of 50 mg/kg bw of cinnamaldehyde by gavage in corn oil. At dose levels of 250 and 500 mg/kg bw, cinnamic acid was detected in blood at concentrations greater than 10 microg/ml, concentrations of cinnamaldehyde was approximately 1 microg/ml. The bioavailability of cinnamaldehyde was calculated to be <20% at both dose levels. In a separate study, rats (3-6 per group) were dosed by gavage with cinnamaldehyde in corn oil at levels of 50, 150, 250, 500, 1000 and 2000 mg/kg bw. A dose-dependent increase in hippuric acid, the major urinary metabolite, occurred 6 hours after gavage and continued over the next 18 hours. The peak hippuric acid occurred at 8 hours after dosing. Since plasma half-life of hippuric acid is reported to be only 15 minutes, the rate of excretion of hippuric acid must be mainly controlled by its rate of formation. Only very small amounts of cinnamic acid were detected in the urine, either as free acid or as the glucuronic acid conjugate. The recovery of hippuric acid in the urine was between 72 and 81% over the dose range of 50 – 500 mg cinnamaldehyde/kg bw (Yuan et al., 1992).

Cinnamic aldehyde, cinnamyl alcohol and cinnamic acid were administered i.p. for 5 days a week for two weeks. Approximately 15% of a dose of 250 mg cinnamaldehyde/kg bw administered to rats was excreted in the urine as two mercapturic acid derivatives, N-acetyl-S-(1-phenyl-3-hydroxypropyl)cysteine and N-acetyl-S-(1-phenyl-2-carboxyethyl)cysteine, in a ratio of four to one. Approximately 9% of a dose of 125 mg cinnamyl alcohol/kg bw was excreted in the urine as N-acetyl-S-(1-phenyl-3-hydroxypropyl)cysteine. Reaction with GSH was not observed when the animals were dosed with cinammic acid, at a dose of 250 mg/kg bw (Delbressine et al., 1981b).

Metabolism of cinnamic acid and other aromatic carboxylic acids

In animals, aromatic carboxylic acids, such as cinnamic acid and its saturated analogue 3-phenylpropionic acid and the structurally related 2-phenylpropionic acid, formed by hydrolysis of the candidate ester [FL-no: 09.364], that enter the cell are converted to acyl CoA esters. The candidate substances 4-hydroxy-3,5-dimethoxycinnamic acid [FL-no: 08.088] and 4-hydroxy-3-methoxycinnamic acid [FL-no: 08.089] (ferulic acid) are expected to behave similarly. All of these acids are expected to be oxidised via the beta-oxidation pathway. For the saturated acids, (e.g. 3-phenylpropionic acid) the first step in this reaction is dehydrogenation of the saturated bond between C2 and C3 of the side chain. For the unsaturated acids (e.g. cinammic acid) this step is not required (the position of the double bond is already between C2 and C3). Cinnamoyl CoA either forms a conjugate with glycine, a reaction catalysed by N-acyl transferase, or undergoes beta-oxidation eventually leading to the formation of benzoyl CoA. The reactions that form benzoic acid from cinnamic acid are reversible but the equilibrium favours formation of the benzoic acid CoA ester. Benzoyl CoA is in turn conjugated with glycine, yielding hippuric acid, or the CoA thioester is hydrolysed to yield free benzoic acid, which is then excreted (Nutley et al., 1994). CoA thioesters of carboxylic acids are obligatory intermediates in amino acid conjugation reactions (Hutt & Caldwell, 1990). The beta-oxidation pathway is the predominant pathway of metabolic detoxication of cinnamic acid in animals.

Dose levels in the range of 0.0005 – 2.5 mmol/kg (approximately 0.074 – 370 mg/kg bw) [^{14}C]- or [^{14}C/ $^{5}H_2$]-cinnamic acid were administered orally to male F344 rats or by intraperitoneal injection to male CD1 mice. In both species, 84-101% of the radioactive dose was recovered within 72 hours with the majority (73-93%) recovered in the urine within 24 hours. The major metabolite was hippuric acid at all dose levels and in both species (69-77% in rats and 44-67% in mice). Several minor metabolites were found in both species, 3-hydroxy-3-phenylpropionic acid, benzoic acid and benzoyl glucuronide. Two metabolites, acetophenone and cinnamoylglycine, the glycine conjugate of cinnamic acid, were only positively identified in the mouse. Results in rats show that with increasing dose the percentage of hippuric acid decreased while the percentages of benzoyl glucuronide and benzoic acid increased. Increased formation of benzoyl glucuronide (0.5-5%) and free benzoic acid (0.4-2%) at dose levels above 0.5 mmol/kg bw provide evidence that saturation of the glycine conjugation pathway occurs at these higher dose levels. The fact that 3-hydroxy-3-phenylpropionic acid was only slightly changed over the dose range (0.2-0.9%) supports the conclusion that the beta-oxidation pathway is not capacity-limited up to 2.5 mmol/kg bw cinnamic acid in the male rat. In mice glycine conjugation of cinnamic acid competes with the beta-oxidation pathway, but only at low dose levels. As dose levels increase from 0.0005 to 2.5 mmol/kg bw, urinary hippuric acid increases, whereas cinnamoylglycine levels decrease from 29 to 2.4%. These results suggest that glycine N-acetyl transferase has high affinity but low capacity for cinnamic acid compared to benzoic acid. At the highest dose, an increase in excreted free benzoic acid (0.8-8.6%) suggests that glycine conjugation of benzoyl CoA is also capacity-limited in mice. At all dose levels, mice excrete a small proportion of benzoyl glucuronide, which suggests that this conjugation reaction is of minimal importance in this species (Nutley et al., 1994).

Eleven adult volunteers received single intravenous doses of cinnamic acid, equivalent to 5 mg/kg bw. Analysis of the blood plasma at 0, 2.5, 5, 10 and 20 minutes after dosing revealed maximum levels of cinnamic acid in healthy volunteers within 2.5 minutes, declining to baseline levels within

20 minutes. Ninety minutes after dosing, urinalysis revealed hippuric acid, cinnamoyl glucuronide and benzoyl glucuronide present in a ratio of 74 : 24.5 : 1.5. In volunteers with renal deficiency, baseline levels were not reached again within 20 minutes after dosing (Quarto di Palo & Bertolini, 1961).

The overall fatty acid oxidation rate of 3-phenylpropionic acid was studied in vitro using male Wistar rat hepatocytes, as part of an experiment to determine the inhibitory effect of valproate (i.e. 2-propylpentanoate) and two of its unsaturated metabolites on the beta-oxidation rate of several long-chain and medium-chain fatty acids. The rate of beta-oxidation of radiolabelled 3-phenyl-propionic acid (prepared as [2,3-^{3}H]-phenylpropionic acid) was determined and expressed in terms of nmol of $^{3}H_2O$ released per mg protein per hour. The incubations made without valproate (or unsaturated metabolites) were considered as controls, and the obtained oxidation rates taken as 100%. A dose-dependent reduction of $^{3}H_2O$ release with increasing doses of valproate demonstrated that beta-oxidation of the phenylpropionic acid did occur (Silva et al., 2001).

III.3.3. Effects of ring substituents on metabolism of cinnamyl derivatives

While ortho-ring substituents may inhibit beta-oxidation *meta*- and *para*-substituents do not significantly impact metabolism via beta-oxidation. The glycine conjugates of *o*-methoxycinnamic and *o*-methoxyphenylpropionic acid are the principal urinary metabolites of *o*-methoxycinnamaldehyde in rats. Relatively large amounts of beta-hydroxylated phenylpropionic acid derivatives were also detected, but only traces of benzoic and hippuric acid derivatives (i.e. products of further beta-oxidation) were excreted. The detection of large amounts of the beta-hydroxylated derivative suggests that this metabolite is not readily further oxidised, possibly due to steric hindrance of the *ortho*-substituent. In male albino rats, *p*-methoxycinnamic acid has been shown to be metabolised primarily to *p*-methoxybenzoic acid and its corresponding glycine conjugate (Solheim & Scheline, 1973). Similar results were reported with 3,4-dimethoxycinnamic acid, which is *meta*- and *para*-substituted (Solheim & Scheline, 1976).

III.3.4. Candidate substances

Because it contains several metabolically active sites, 4-hydroxy-3-methoxycinnamic acid [FL-no: 08.089] (ferulic acid) may be transformed through various pathways to a large number of metabolites. The number of metabolites and their levels would depend on several factors including the dose administered, route of administration and species of the animal. Various experimental data substantiate that ferulic acid qualitatively has the same metabolic fate as caffeic acid (3,4-dihydroxycinnamic acid) (Bourne & Rice-Evans, 1998; Scheline, 1991).

The same ten 3-hydroxyphenyl and 3-methoxy-4-hydroxyphenyl acids were excreted in human urine following the ingestion of caffeic or ferulic acids (Shaw & Trevarthen, 1958; Booth et al., 1957).

When rats were fed caffeic acid, 3-hydroxyphenylpropionic acid (*m*-HPPA) was the major metabolite although some ferulic acid, dihydroferulic acid and traces of 1-carbon side chain compounds could be detected. Thus, the rat metabolised most of the caffeic acid to compounds having a 3-carbon side chain, and only small amounts of *m*-hydroxyhippuric acid (*m*-HHA), vanillic acid and vanilloylglycine were excreted. When low doses of caffeic acid were administered to rats,

the only conspicuous metabolite in the urine was *m*-HPPA. Only traces of ferulic acid were detected. After i.p. injection of caffeic acid (as sodium salt) the same metabolites were observed as after oral administration. There is indication that the preferred route of caffeic acid metabolism is to *m*-HPPA in rats and to *m*-HHA in humans. However, if the involved enzyme systems become overloaded other subordinate pathways come into play and a greater assortment of degradation products appears (Booth et al., 1957). When ferulic acid [FL-no: 08.089], being one of the metabolites found after administration of caffeic acid, was fed to rats, it was found to be a precursor for principally 3-hydroxyphenylpropionic acid (*m*-HPPA), and the hydroxymethoxy derivatives feruloylglycine ([3-(4-hydroxy-3-methoxyphenyl)-acryloylamino]-acetic acid), dihydro ferulic acid, vanillic acid and vanilloylglycine ((4-hydroxy-3-methoxybenzoylamino)-acetic acid) (Booth et al., 1957).

3-Hydroxyphenylpropionic acid (*m*-HPPA) was the main urinary metabolite when ferulic acid [FL-no: 08.089] was administered via intraperitoneal injection to female rats. Vanillic acid was also excreted (Teuchy & Van Sumere, 1971). The conversion of ferulic acid to vanillic acid by rat liver homogenates has also been reported (Dirscherl & Brisse, 1966).

The intestinal flora may be of considerable importance in determining the metabolic fate of phenolic acids. When a number of hydroxyphenylacetic acids as well as hydroxyphenylpropionic and cinnamic acids were incubated anaerobically with a mixed culture of rat caecal microorganisms four reactions were found: reduction of a double bond, dehydroxylation, decarboxylation and demethylation. Reduction of a double bond was observed in all the cinnamic acid derivatives tested. And all compounds with 3,4-dihydroxyphenyl substitution underwent dehydroxylation at the *p*-position. The reduced metabolites were the major metabolites observed of these compounds. Decarboxylation was only observed when a free *p*-hydroxy group was present, except for hydrocinnamic and hydroferulic acid, which were resistant to decarboxylation. The metabolism of ferulic acid to 3-hydroxyphenylpropionic acid (*m*-HPPA) involves a reduction of the double bond to yield dihydroferulic acid, then a demethylation to yield dihydrocaffeic acid and finally the dehydroxylation to *m*-HPPA. The end product *m*-HPPA as well as the intermediates dihydroferulic acid and dihydrocaffeic acid were seen when ferulic acid was incubated with the rat caecal extract. In some of the samples the decarboxylated metabolites 4-vinylcatechol and 4-ethylcatechol were observed as further products of ferulic acid metabolism by rat intestinal flora (Scheline, 1968b).

As there is evidence that the dehydroxylation is a reaction which occurs only in the intestine of animals and not in other tissues, the presence of dehydroxylated metabolites of caffeic acid and ferulic acid in the urine after i.p. injection to rats might best be explained by biliary excretion of caffeic acid and ferulic acid, or of a metabolite which is capable of being metabolised to *m*-HPPA by the intestinal flora (Scheline, 1968b). More than 40% of an i.v. dose (approx. 45 mg/kg) of ferulic acid was found to be excreted in the bile within 6 hours, predominantly as the glucuronide (Plummer, 1977). Likewise, extensive biliary excretion of ferulic acid in rats after i.v. or intradermal application (up to 30% of the dose) was reported compared with a small value (3-5%) seen with *p*- or *m*-coumaric or caffeic acid (Westendorf & Czok, 1978). Thus, administration of ferulic acid to rats either orally or by injection will lead to a qualitatively similar pattern of metabolism since the biliary ferulic acid glucuronide (feruloyl glucuronide) is readily hydrolyzed and further metabolised by the intestinal bacteria.

III.4 Conclusions for absorption, distribution, metabolism and elimination

Information on kinetics is very limited on candidate substances and conclusions are therefore mainly based on supporting substances. The candidate substances are structurally closely related to the supporting chemicals and are expected to behave similarly.

The supporting substance cinnamic acid, its methyl ester and other structurally related derivatives as well as the saturated analogue 3-phenylpropanoic acid and its derivatives, have been shown to be rapidly absorbed from the gut. It is anticipated that the structurally related ethyl ester of 2-phenylpropionic acid [FL-no: 09.364] also is absorbed.

Esters of cinnamic acid and structurally related aromatic esters have been shown to be hydrolysed to the component carboxylic acid and alcohol. It is therefore anticipated that the candidate ester pentyl cinnamate [FL-no: 09.735] is hydrolysed to cinnamic acid and pentyl alcohol, and the candidate esters 3-phenylpropyl benzoate and 3-phenylpropyl 3-phenylpropionate [FL-no 09.836 and 09.837] would be hydrolysed to benzoic acid and 3-phenylpropanoic acid, respectively, and in both cases to the aromatic alcohol 3-phenylpropanol. It is anticipated that the candidate ester 3-phenylpropyl butyrate [FL-no: 09.690] would be hydrolysed to butanoic acid and 3-phenylpropanol and ethyl 2-phenylpropionate [FL-no: 09.364] to 2-phenylpropionic acid and ethanol.

3-Phenylpropanol formed by the hydrolysis of candidate esters [FL-no: 09.690, 09.836 and 09.837] may subsequently be oxidised to 3-phenylpropanoic acid. The structurally related candidate aromatic primary alcohol [FL-no: 02.173] and aromatic aldehyde [FL-no: 05.156] are similarly expected to be oxidised to 3-(4-methoxyphenyl)propanoic acid and 3-(4-hydroxy-3-methoxyphenyl)propanoic acid, respectively.

Aromatic carboxylic acids, such as cinnamic acid and its saturated analogue 3-phenylpropanoic acid, are expected to be primarily converted to CoA esters and either to form conjugates with glycine or to undergo further beta-oxidation leading to the formation of benzoyl CoA. *Meta*- and *para*-substituents, in relation to the three carbon side chain which are present in [FL-no: 02.173 and 05.156] have no significant effect on the metabolism via beta-oxidation. Benzoyl CoA that is formed from these reactions may be conjugated with glycine and excreted as hippuric acid or may be hydrolysed to yield free benzoic acid which is then excreted. The same reactions are predicted to occur for the substituted benzoic acid CoA esters.

It is concluded that the nine candidate substances in this flavouring group may be expected to be absorbed and metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for one candidate substances of the present flavouring group evaluation from chemical group 22, and for 18 supporting substances evaluated by JECFA at the 55th meeting. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(3-Phenyl-1-propanol [02.031])	Rat	NR	Oral	2300	(Moreno, 1976p)	Confidence limits of 1500-3100 mg/kg bw, 4 dose groups of 10 animals, only summary available.
	Rat	M, F	Oral	2250	(Wong & Weir, 1971a)	Value in µl/kg bw, 4 dose groups of 5 animals used, confidence limits of 1680-3000 µl/kg bw.
(3-Phenylpropionaldehyde [05.080])	Rat	NR	Oral	>5000	(Russell, 1973d)	One group of 10 animals received 5000 mg/kg, mortality 4/10, only summary available.
(Cinnamic acid [08.022])	Rat	NR	Oral	3570	(Levenstein, 1976e)	Value in µl/kg bw , confidence limits of 3070-4140 µl/kg bw, 3 dose groups (3250-5000 µl/kg bw) of 10 animals, only summary available.
(3-Phenylpropionic acid [08.032])	Mouse	NR	Feed	>913[1]	(Schafer & Bowles, 1985)	Unusual study design. Study not considered valid for the derivation of an LD50 value.
	Mouse	NR	Oral	>1600[2]	(Schafer & Bowles, 1985)	Unusual study design. Study not considered valid for the derivation of an LD50 value.
(3-Phenylpropyl acetate [09.032])	Rat	NR	Oral	4700	(Moreno, 1973j)	Confidence limits of 3840-5560 mg/kg bw, 4 dose groups (2220-7500 mg/kg bw) of 10 animals, only summary available.
(3-Phenylpropyl formate[09.084])	Rat	NR	Oral	4090	(Levenstein, 1975e)	Value in µl/kg bw, confidence limits of 3490-4780 µl/kg bw, 3 dose groups of 10 animals, only summary available.
(3-Phenylpropyl propionate [09.138])	Rat	NR	Oral	>5000	(Moreno, 1977p)	One group of 10 animals received 5000 mg/kg, mortality 2/10, only summary available.
(3-Phenylpropyl isobutyrate [09.428])	Rat	NR	Oral	>5000	(Levenstein, 1975f)	One group of 10 animals received 5000 mg/kg, mortality 1/10, only summary available.
(Propyl cinnamate [09.731])	Mouse	NR	Oral	7000	(Draize et al., 1948)	Published study. Value in µl/kg bw, 6 dose groups of 10 animals.
(Isopropyl cinnamate [09.732])	Rat	NR	Oral	>5000	(Moreno, 1982h)	One group of 10 animals received 5000 mg/kg,

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
						mortality 0/10, only summary available.
(Butyl cinnamate [09.733])	Rat	NR	Oral	>5000	(Moreno, 1977r)	One group of 10 animals received 5000 mg/kg, mortality 0/10, only summary available.
(Isobutyl cinnamate [09.734])	Rat	NR	Oral	>5000	(Levenstein, 1975g)	One group of 10 animals received 5000 mg/kg, mortality 2/10, only summary available.
Pentyl cinnamate [09.735]	Rat	NR	Oral	>5000	(Moreno, 1974i)	One group of 10 animals received 5000 mg/kg, mortality 0/10, only summary available.
(Linalyl cinnamate [09.736])	Rat	M, F	Gavage	9960	(Jenner et al., 1964)	Published study. Confidence limits of 8230-12050 mg/kg bw, groups of 5 M and 5 F.
(Benzyl cinnamate [09.738])	Rat	M, F	Gavage	3280	(Wolven & Levenstein, 1972)	Confidence limits of 2620-4100 mg/kg bw, 4 doses (2000-5000 mg/kg bw) were given to groups of 5 M and 5 F.
(Methyl cinnamate [09.740])	Rat	M, F	Oral	2610	(Wong & Weir, 1971b)	Confidence limits of 2000-3410 mg/kg bw, 6 doses up to 6000 mg/kg bw) were given to groups of 5 M and 5 F.
(Phenethyl cinnamate [09.743])	Rat	NR	Oral	5000[3]	(Moreno, 1975k)	One group of 10 animals received 5000 mg/kg, mortality 5/10, only summary available.
	Mouse	NR	Oral	>5000	(Levenstein, 1975h)	5 animals received 5000 mg/kg, mortality 0/4, only summary available.
(3-Phenylpropyl cinnamate [09.745])	Rat	NR	Oral	>5000	(Keating, 1972d)	One group of 10 animals received 5000 mg/kg, mortality 0/10, only summary available.
(Methyl 3-phenylpropionate [09.746])	Rat	NR	Oral	4200	(Moreno, 1981b)	Confidence limits of 3100-5700 mg/kg bw, 4 dose groups (1200-5000 mg/kg bw) of 10 animals, only summary available.

M = Male; F = Female.

NR = Not reported.

1 Amount of chemical ingested during a feed reduction test that killed or did not kill not more than 50% of the test mice.

2 Approximate lethal dose.

3 Approximate LD50 value.

Subacute / subchronic / chronic / carcinogenic toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 22 and for four supporting substances evaluated by JECFA at the 55th meeting. The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No/Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
4-Hydroxy-3-methoxycinnamic acid [08.089]	Rat; M 5	Diet	0, 2% in the diet (0, 2000 mg/kg bw/d)[4]	4 weeks	2000 [1,3]	(Hirose et al., 1987)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
(Ethyl cinnamate [09.730])	Rat; M 12	Oral (gavage)	80 mg/kg bw/d	4 months	80[1]	(Zaitsev & Rakhmanina, 1974)	Study published in Russian (only abstract in English available). No control group used. The study is considered not valid.
	Rat; M, F 24	Diet	0, 3 mg/kg bw/d	12 weeks	3[1, 2]	(Trubek Laboratories, Inc., 1958b)	Unpublished non-GLP study. Experimental details and results insufficiently reported. The validity of the study cannot be evaluated. Study not designed to determine a NOAEL of a single flavouring substance.
(Linalyl cinnamate [09.736])	Rat; M, F 20	Diet	0, 50, 125, 500 mg/kg bw/d	17 weeks	500[1]	(Hagan et al., 1967)	Published non-GLP study of acceptable quality. Results not reported in detail but summarized in table.
(Benzyl cinnamate [09.738])	Rat; M, F 20	Diet	0, 50, 500 mg/kg bw/d	19 weeks	500[1]	(Hagan et al., 1967)	Published non-GLP study of acceptable quality. Results not reported in detail but summarized in table.
(Methyl cinnamate [09.740])	Rat; M, F 24	Diet	0, 3 mg/kg bw/d	12 weeks	3[1, 2]	(Trubek Laboratories, Inc., 1958b)	Unpublished non-GLP study. Experimental details and results insufficiently reported. The validity of the study cannot be evaluated. Study not designed to determine a NOAEL of a single flavouring substance.

M = Male; F = Female.

1 This study was performed at either a single dose or multiple dose levels that produced no adverse effects. Therefore, this dose level is the highest dose tested that produced no adverse effects.

2 The test substance was administered as a component of a mixture composited in proportion to the following use levels: cinnamic aldehyde 897 mg/kg, methyl cinnamate 25 mg/kg, ethyl cinnamate 25 mg/kg, cinnamyl cinnamate 25 mg/kg and alpha-methyl cinnamic aldehyde 25 mg/kg. Mixture was incorporated into the diet to provide a daily intake of 100 mg of flavour per kg bw.

3 Study evaluated the induction of forestomach lesions in rats for four weeks.

4 Calculated based on a bw of 250 g and a daily food intake of 12 g.

Developmental and reproductive toxicity data are available for none of the candidate substances of the present flavouring group evaluation from chemical group 22 but for one the supporting substance evaluated by JECFA at the 55th meeting. Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Durations	Species/Sex No/Group	Route	Dose levels	NOAEL (mg/kg/day) Including information on possible maternal toxicity	Reference	Comments
(Cinnamic acid [08.022])	Developmental Toxicity: Throughout pregnancy	Rat; F 14 - 15	Oral	0, 5, 50 mg/kg bw/day	Maternal: 50 Foetal: 50	(Zaitsev & Maganova, 1975)	Published non-GLP study. Translation of original Russian text available. Limited report of experimental details and results.

In vitro mutagenicity/genotoxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 22 and for six supporting substances evaluated by JECFA at the 55th meeting. Supporting substances are listed in brackets.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(3-Phenylpropionaldehyde [05.080]) syn. 3-Phenylpropanal	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmoles/plate (403 µg/plate)[14]	Negative[1]	(Florin et al., 1980)	Published non-GLP study. Qualitative screening in a spot-test only. Precipitates of substance reported. Limited report of experimental details and results. Validity of the study cannot be evaluated. Study not considered adequate for the evaluation of mutagenic activity.
	Sister chromatid exchange	Chinese hamster ovary cells	0, 1.0, 3.3, 10, 33.3, 100µM (0, 0.134, 0.443, 1.34, 4.43, 13.4 µg/ml)[7, 14]	Negative[2, 8, 15]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs.
(Cinnamic acid [08.022])	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1-1000 µg	Negative[1]	(Lijinsky & Andrews, 1980)	Published non-GLP study of acceptable quality. Limited report of detailed results (for controls only).
	Rec assay	*B. subtilis* M45 (rec-), H17 (rec+)	25 µg/plate	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec-), H17 (rec+)	2.0 mg/plate (2000 µg/plate)	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	0, 1.0, 3.3, 10, 33.3, 100 µM (0, 0.148, 0.489, 1.482, 4.933, 14.82 µg/ml)[4, 7]	Negative[2, 8, 9]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs.
4-Hydroxy-3,5-dimethoxycinnamic acid [08.088]	Mutation assay	*E. coli* B/r WP2	1000 µg/plate	Negative[2, 5]	(Shimoi et al., 1985)	Published non-GLP study. Study designed for the determination of effects on UV-induced mutagenesis. Experimental details of the assessment of direct mutagenic activity not reported and results not shown. Thus, the validity of these data cannot be evaluated.
4-Hydroxy-3-methoxycinnamic acid [08.089]	Ames reverse mutation assay	*S. typhimurium* TA98, TA100	NR[6]	Negative[1, 3]	(Matsuda et al., 1992)	Published non-GLP study. Limited report of experimental details and results. Validity of the study cannot be evaluated. Study designed for the determination of ozonation products of 4-hydroxy-3-methoxy-cinnamic acid (and other structural components of humic substances). Thus only results of negative control (not ozonated) are of relevance in this evaluation.
	Mutation assay	*E. coli* B/r WP2	1000 µg/plate	Negative[2, 5]	(Shimoi et al., 1985)	Published non-GLP study. Study designed for the

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						determination of effects on UV-induced mutagenesis. Experimental details of the assessment of direct mutagenic activity not reported and results not shown. Thus, the validity of these data cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	0, 3.3, 10, 33.3, 100, 333 µM (0, 0.641, 1.94, 6.41, 19.4, 64.1 µg/ml)[7, 12]	Negative[2, 8, 9, 13]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-, UV- and X-ray-induced SCEs.
(Ethyl cinnamate [09.730])	Ames reverse mutation assay (preincubation method)	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537,	up to 5000 µg/plate[17]	Negative[1]	(Ishidate et al., 1984)	Published non-GLP study of acceptable quality.
	Chromosomal aberration assay	Chinese hamster fibroblasts	up to 63 µg/ml[18]	Equivocal[2, 19] Negative[2, 19]	(Ishidate et al., 1984)	Published non-GLP study of limited quality.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µg/plate	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	0, 1.0, 3.3, 10, 33.3 µM (0, 0.176, 0.581, 1.76, 5.81 µg/ml) [11, 7]	Negative[2, 8, 9]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs.
(Benzyl cinnamate [09.738])	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmoles/plate (715 µg/plate)[16]	Negative[1]	(Florin et al., 1980)	Published non-GLP study. Qualitative screening in a spot-test only. Precipitates of substance reported. Limited report of experimental details and results. Validity of the study cannot be evaluated. Study not considered adequate for the evaluation of mutagenic activity.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	1.0 mg/disk (1000 µg/plate)	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Methyl cinnamate [09.740])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µg/plate	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	0, 1.0, 3.3, 10, 33.3, 100 µM (0, 0.162, 0.535, 1.62, 5.40, 16.2 µg/ml)[10, 7]	Negative[2, 8, 9]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs.
(Cyclohexyl cinnamate [09.744])	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	up to 3600 µg/plate[20]	Negative[1]	(Wild et al., 1983)	Published non-GLP study. No detailed results reported. However, as experimental details and evaluation criteria including results of positive controls are sufficiently reported the study is considered valid.

NR = Not reported.

1 With and without S9 metabolic activation.

2 Without S9 metabolic activation.

3 Ozonated samples gave a positive result (more than three times compared to spontaneous mutation) with tester strain TA100 with metabolic activation and weakly positive result (1.5-3 times) without metabolic activation possibly due to formed ozonation products (aldehydes, ketones and carboxylic acids such as formaldehyde, acetaldehyde, glyoxal and methylglyoxal as identified in the same study after ozonation of p-hydroxybenzaldehyde).

4 Calculated based on molecular weight = 148.15.

5 Negative result reported for both direct mutagenic activitäy and enhancement of UV-induced mutagenesis.

6 Unquantified samples of 4-hydroxy-3-methoxy-cinnamic acid were ozonated at a ratio of sample to ozone of 1:0 (control), 1:0.5, 1:1 and 1:6 (by weight) and then tested for mutagenicity.

7 The highest concentration was reported to be toxic.

8 The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used.

9 Posttreatment of mitomycin-treated cells with the substance increased the frequency of induced SCEs in a dose-related manner. The effect was statistically significant ($p<0.001$) at the two highest nontoxic concentrations.

10 Calculated based on molecular weight = 162.15.

11 Calculated based on molecular weight = 176.21.

12 Calculated based on molecular weight = 194.19.

13 The frequency of SCEs induced by UV was significantly increased by treatment with 4-hydroxy-3-methoxy-cinnamic acid at 10 ($0.001<p<0.01$), 33.3 and 100 μM ($p<0.001$) in a dose-related manner. On the contrary, X-ray induced SCEs were significantly reduced by treatment with 4-hydroxy-3-methoxy-cinnamic acid at 10 ($0.01<p<0.05$), 33.3 and 100 μM ($p<0.001$). The effect was also dose-related.

14 Calculated based on molecular weight = 134.17.

15 Posttreatment of mitomycin-treated cells with the substance did not influence the frequency of induced SCEs.

16 Calculated based on molecular weight = 238.27.

17 Six different concentrations used (single concentrations not reported).

18 Three different doses used (single doses not reported).

19 Negative result with respect to chromosomal aberrations; equivocal result considering the observed polyploidization effect.

20 Five different concentrations used (single concentrations not reported).

No *In vivo* mutagenicity/genotoxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 22 or for the supporting substances evaluated by JECFA at the 55th meeting.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test system	Test Object	Dose	Result	Reference	Comments

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