

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

**Flavouring Group Evaluation 14 (FGE.14):
Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters.
From chemical group 15
(Commission Regulation (EC) No 1565/2000 of 18 July 2000)**

Adopted on 27 April 2005

SUMMARY

The Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food 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 Panel is asked to evaluate ten flavouring substances in the Flavouring Group Evaluation FGE.14, using the procedure as referred to in the Commission Regulation EC No 1565/2000. These ten flavouring substances belong to chemical group 15, Annex I of the Commission Regulation EC No 1565/2000.

The present Flavouring Group Evaluation deals with seven phenethyl alcohol derivatives (alcohol, esters and acetals), one phenethyl aldehyde derivative and two phenylacetates.

Two of the ten flavouring substances possess a chiral centre and one flavouring substance possesses three chiral centres. The substances have been evaluated irrespective of their chirality.

All ten flavouring substances are classified into structural class I.

Eight of the substances 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 ten flavouring substances have intakes in Europe from 0.012 to 1.9 microgram/*capita*/day which are well below the threshold of concern for structural class I of 1800 microgram/person/day.

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All ten flavouring substances are expected to be metabolised to innocuous substances.

Overall, the genotoxicity data available are not sufficient to evaluate the genotoxicity adequately, however, the data available on candidate and supporting substances do not give rise to concern with respect to genotoxicity of the ten candidate substances in this flavouring group evaluation. Consideration was given to ethanol and acetaldehyde, two potential hydrolysis products of the acetals [FL-no: 06.078 and 06.080]. Because of the natural occurrence in food and the endogenous formation in humans of considerably larger amounts of these compounds, their formation from hydrolysis of the acetals was not considered to be of safety concern with respect to genotoxicity at their estimated levels of intakes, based on the MSDI approach.

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 ten substances would not give rise to safety concerns at 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 3700 microgram/person/day for the ten flavouring substances from structural class I. Thus, the intakes were above the threshold of concern for structural class I of 1800 microgram/person/day, except for one candidate substance [FL-no: 05.159]. The one substance [FL-no: 05.159], which has an mTAMDI intake below the threshold of concern for structural class I, is also expected to be metabolised to innocuous products.

Thus, for nine of the ten flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for the structural class to which the flavouring substance has been assigned. Therefore, for these nine substances [FL-no: 02.166, 06.078, 06.080, 09.201, 09.620, 09.684, 09.685, 09.686, and 09.761] 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 of the ten flavouring 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 all ten flavouring substances, except that information on chirality is missing for two and information on the structure of the phenethyl moiety is lacking for four of the substances. Thus, the final evaluation of the materials of commerce cannot be performed for five substances [FL-no: 06.078, 09.201, 09.620, 09.684, and 09.686], pending further information.

KEYWORDS

Flavourings, safety, phenethyl alcohols, phenyl ethyl alcohols, phenylacetic acid, ester.

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Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15**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 others 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 2004/357/EC (EC, 2004). 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).

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).

TERMS OF REFERENCE

EFSA is requested to carry out a risk assessment on flavouring substances prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000).

ASSESSMENT**1. Presentation of the Substances in the Flavouring Group Evaluation 14****1.1. Description**

The present Flavouring Group Evaluation, FGE.14, using the procedure as referred to in the Commission Regulation EC No 1565/2000 (EC, 2000) (The Procedure –shown in schematic form in Annex I), deals with seven phenethyl alcohol derivatives (alcohol, esters and acetals), one phenethyl aldehyde derivative and two phenylacetates. These ten flavouring substances belong to chemical group 15, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000).

The ten flavouring substances under consideration, with their chemical names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1 and 2a. This group of substances (candidate substances) includes one phenethyl alcohol [FL-no: 02.166], four phenethyl esters [FL-no: 09.686, 09.201, 09.685, and 09.684], two phenethyl acetals [FL-no: 06.080 and 06.078], one phenylacetaldehyde [FL-no: 05.159], and two phenylacetates [FL-no: 09.761 and 09.620].

The ten flavouring substances (candidate substances) are closely related structurally to 37 flavouring substances (supporting substances) evaluated at the 59th JECFA meetings (JECFA, 2002c) in the group of “Phenethyl alcohol, aldehyde, acid and related acetals and esters”.

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The hydrolysis products of the candidate substances are listed in Table 2b, and the supporting substances are listed in Table 3.

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.).

Two of the ten candidate substances possess a chiral centre [FL-no: 06.080 and 09.686]. One candidate substance [FL-no: 09.620] possesses three chiral centres. The substances have been presented without any indication whether the commercial flavouring substance was dominated by one optical isomer or the other (see Table 1).

Due to the presence and the position of a double bond, one of the candidate substances [FL-no: 09.684] can exist as geometrical isomers. But the geometrical isomeric form is given by the name.

1.3. Natural Occurrence in Food

Eight out of ten candidate substances have been reported to occur in beer, various types of alcoholic beverages, cranberries, lamb's lettuce, essential oils, and wort. Quantitative data on the natural occurrence in foods have been reported for six of these substances.

These reports are:

- 2-(4-Hydroxyphenyl)ethan-1-ol [FL-no: 02.166]: Up to 59 mg/kg in beer, 10 mg/kg in cider, 4.9 mg/kg in sherry, 101 mg/kg in sake, up to 0.29 mg/kg in cranberry, and 0.02 mg/kg in wort.
- 1,1-Diphenethoxyethane [FL-no: 06.078]: 0.03 mg/kg in white wine.
- 1-Ethoxy-1-(2-phenylethoxy)ethane [FL-no: 06.080]: Up to 0.06 mg/kg in white wine.
- Phenethyl valerate [FL-no: 09.201]: 0.02 mg/kg in beer, up to 1200 mg/kg in eucalyptus oil, and 1 mg/kg in lamb's lettuce.
- 2-Phenethyl decanoate [FL-no: 09.685]: 0.2 mg/kg in rum.
- Pentyl phenylacetate [FL-no: 09.761]: 50 mg/kg in peppermint oil.

Two of the substances, *p*-methoxyphenylacetaldehyde [FL-no: 05.159] and phenethyl crotonate [FL-no: 09.684] have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

2. Specifications

Purity criteria for the ten candidate substances have been provided by the flavouring industry (EFFA, 2003k).

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Judged against the requirements in Annex II of Commission Regulation EC No 1565/2000 (EC, 2000), the information is adequate for the ten candidate substances. However, information on chirality is needed for two candidate substances [FL-no: 09.620 and 09.686] and information on the structure of the phenethyl moiety is lacking for four substances [FL-no: 06.078, 09.201, 09.684, and 09.686] (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 “Maximized 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, 2004).

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.

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Average *per capita* intake MSDI is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population¹ (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).

In the present Flavouring Group Evaluation (FGE.14) the total annual volume of production of the ten candidate substances for use as flavouring substances in Europe has been reported to be approximately 40 kg (EFFA, 2003i) and for 37 supporting substances approximately 17000 kg (cited by JECFA (JECFA, 2003a)).

On the basis of the annual volumes of production reported for the ten candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a). More than 90 % of the total annual volume of production for the candidate substances (EFFA, 2003i) is accounted for by four flavourings: menthyl phenylacetate [FL-no: 09.620], phenethyl crotonate [FL-no: 09.684], phenethyl lactate [FL-no: 09.686], and pentyl phenylacetate [FL-no: 09.761]. The estimated daily *per capita* intakes of these candidate substances from use as a flavouring substance are 1.5, 0.73, 0.24 and 1.9 microgram, respectively. 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 a certain amount of flavourable foods and beverages per day.

For the present evaluation of the ten candidate substances, information on food categories and normal and maximum use levels^{2,3} were submitted by the Flavour Industry (EFFA, 2002a; EFFA, 2001c). The ten candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation 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.

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.

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).

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

According to the Flavour Industry the normal use levels for the ten 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, 2002a; EFFA, 2003k).

The mTAMDI values for the ten candidate substances from structural class I (see Section 5) range from 1600 to 3700 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

The six esters [FL-no: 09.686, 09.201, 09.685, 09.684, 09.761, and 09.620] included in this Flavouring Group Evaluation are expected to be hydrolysed to the corresponding carboxylic acids and alcohols (see Table 2), based on the evaluation of supporting substances (see Annex III).

It is anticipated that the two candidate acetals 1-ethoxy-1-(2-phenylethoxy)ethane [FL-no: 06.080] and 1,1-diphenethoxyethane [FL-no:06.078] would undergo rapid hydrolysis in the gastric environment (see Table 2 and Annex III).

The carboxylic acids resulting from hydrolysis of the six candidate esters: lactic acid, valeric acid, decanoic acid and crotonic acid are all expected to be metabolised via common pathways, including beta-oxidation and citric acid cycle. From the hydrolysis of the two candidate acetals acetaldehyde is obtained, which is expected to be oxidised to acetic acid by aldehyde dehydrogenase. Acetic acid will follow the same metabolism as the carboxylic acids above.

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The metabolic elimination of alcohols in experimental animals and man occurs primarily by two pathways: (1) oxidation to the aldehyde and subsequently to the corresponding carboxylic acid, and (2) conjugation of the alcohol with glucuronic acid.

The candidate alcohol, 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166], is absorbed in the intestine of humans, and mainly excreted in the urine in conjugation with glucuronic acid. The candidate aldehyde, *p*-methoxyphenylacetaldehyde [FL-no: 05.159], is oxidized to the corresponding carboxylic acid and conjugated to amino acids like glutamine and glycine before excretion in the urine.

In addition, menthol is released after hydrolysis of menthyl phenylacetate [FL-no: 09.620]. Menthol is mainly conjugated with glucuronic acid and excreted by the bile in rats (Yamaguchi et al., 1994). Oxidation of the methyl and isopropyl substituents of the cyclohexyl ring may occur before conjugation with glucuronic acid. Low levels of oxidation products were found in the urine, but no unchanged menthol was detected in the urine, faeces or bile (Yamaguchi et al., 1994).

Phenethyl alcohol and phenylacetic acid are two of the major hydrolysis products. Phenethyl alcohol is oxidised first to the aldehyde and subsequently to phenylacetic acid. Phenylacetic acid is an endogenous end-product of phenylalanine metabolism and is present in human urine as a conjugate (Seakins, 1971). The types of conjugates formed from phenylacetic acid are both dose-dependent and species-specific. The major metabolic options available to phenylacetic acid are conjugation with glucuronic acid, glycine, taurine or glutamine, or elimination as the free acid. In humans, phenylacetic acid is mainly excreted in conjugation with glutamine.

In summary, the ten candidate substances can be predicted 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. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach (see Section 6).

For the safety evaluation of the ten candidate substances from chemical group 15 the Procedure as outlined in Annex I was applied. The stepwise evaluations of the ten substances are summarised in Table 2a.

Step 1

All ten candidate substances of chemical group 15 [FL-no: 02.166, 05.159, 06.078, 06.080, 09.201, 09.620, 09.684, 09.685, 09.686 and 09.761] are classified in structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

Step 2 requires consideration of whether detoxification pathways are available to metabolise the substances, at the expected levels of intake, to innocuous products.

The ten candidate substances are expected to be metabolised to innocuous products and accordingly their evaluations proceed via the A-side of the Procedure scheme (Annex I).

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Step A3

The ten candidate substances have estimated European daily *per capita* intakes (MSDI) from 0.012 to 1.9 microgram (Table 2a). The intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

Based on results of the safety evaluation sequence these ten candidate substances proceeding via the A-side of the Procedure do not pose a 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 ten candidate substances in structural class I based on the mTAMDI range from 1600 to 3700 microgram/person/day. For one of the substances [FL-no: 05.159] the mTAMDI is below the threshold of concern 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 nine candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

FL-no	EU Register name	MSDI ($\mu\text{g}/\text{capita}/\text{day}$)	mTAMDI ($\mu\text{g}/\text{person}/\text{day}$)	Structural class	Threshold of concern ($\mu\text{g}/\text{person}/\text{day}$)
02.166	2-(4-Hydroxyphenyl)ethan-1-ol	0.12	2100	Class I	1800
05.159	p-Methoxyphenylacetaldehyde	0.037	1600	Class I	1800
06.078	1,1-Diphenethoxyethane	0.012	3700	Class I	1800
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane	0.012	3700	Class I	1800
09.201	Phenethyl valerate	0.012	3700	Class I	1800
09.620	Menthyl phenylacetate	1.5	3700	Class I	1800
09.684	Phenethyl crotonate	0.73	3700	Class I	1800
09.685	2-Phenethyl decanoate	0.037	3700	Class I	1800
09.686	Phenethyl lactate	0.24	3700	Class I	1800
09.761	Pentyl phenylacetate	1.9	3700	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 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 total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDIs for individual substances.

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On the basis of the reported annual production volumes in Europe (EFFA, 20031), the total estimated daily *per capita* intake as flavourings of the ten candidate flavouring substances assigned to structural class I from chemical group 15 is 4.6 microgram, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day.

The ten candidate substances are structurally related to 37 supporting substances evaluated by JECFA at its 59th meeting (JECFA, 2003a). The total estimated combined intake (in Europe) of the candidate and the supporting substances, all assigned to structural class I, is 2006 microgram/*capita*/day, which exceeds the threshold of concern for the corresponding structural class (1800 microgram/ person/day).

However, a major contribution (60 %) was provided by one supporting substance, namely 2-phenylethan-1-ol [FL-no: 02.019] (1200 microgram/*capita*/day). 2-Phenylethan-1-ol is oxidised to phenylacetaldehyde, which is further oxidised to phenylacetic acid, an endogenous end-product of phenylalanine metabolism. Phenylacetic acid is excreted in human urine as a conjugate. 2-Phenylethan-1-ol is accordingly not expected to be of safety concern at the estimated level of intake as most of it is found to be excreted within 24 hours after administration of doses in the milligram range and as high levels of glutamine available for conjugation allow metabolic pathways to cope with high levels of endogenously formed phenylacetic acid in humans. Excluding the major contributor (2-phenylethan-1-ol), the estimated combined intake (in Europe) for the candidate and supporting substances belonging to structural class I would be approximately 800 microgram/*capita*/day, which does not exceed the threshold of concern for the corresponding structural class (1800 microgram/person/day).

8. Toxicity

8.1. Acute Toxicity

Data are available for two candidate substances [FL-no: 06.080 and 09.620]. Oral LD₅₀ values from two studies in rats were >5000 mg/kg bw for both substances.

Twenty-four supporting substances were tested for acute oral toxicity in mouse, rat and guinea pig. The LD₅₀ values ranged from 400 mg/kg bw to approximately 15000 mg/kg bw.

All acute toxicity studies are summarised in Annex IV, Table IV.1. The magnitudes of the LD₅₀ values indicate that the oral acute toxicity is low for the candidate substances and supporting substances.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

No data on oral, subacute, subchronic and chronic toxicity and no carcinogenicity studies are available for the candidate substances. For four supporting substances there are oral subchronic toxicity data [FL-no: 02.019, 05.044, 09.031, and 09.407] (see Annex IV, Table IV.2).

8.3. Developmental / Reproductive Toxicity Studies

There are no developmental or reproductive studies available for the candidate substances. For two supporting substances [FL-no: 02.019 and 08.038] there are developmental/reproductive toxicity data (See Annex IV, Table IV.3).

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8.4. Genotoxicity Studies

Valid *in vitro* mutagenicity and/or genotoxicity data are available for one candidate [FL-no: 02.166] and for two supporting substances [FL-no: 02.019 and 09.784]. There are neither *in vivo* mutagenicity/genotoxicity data available for the candidate substances of the present flavouring group evaluation nor for the supporting substances previously evaluated by JECFA.

Valid *in vitro* and limited *in vivo* mutagenicity data are available for isoeugenyl phenylacetate, a phenyl acetate ester structurally related to the candidate substances in this evaluation (Wild et al., 1983).

For the candidate substance 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166] there are data available from a Comet assay in oxidative stress sensitive PC human prostate cancer cells (PC3) in which the substance at any of the concentrations tested did not increase the value of oxidative DNA damage (DNA strand breaks) as compared to control cells. On the contrary, at relatively high concentrations the substance was found to decrease DNA-damage induced by hydrogen peroxide. However, results indicated that the substance induced lipid peroxidation and decreased the antioxidant capacity of the cells. These effects on enzymes may be attributed to a pro-oxidant activity of 2-(4-hydroxyphenyl)ethan-1-ol (Quiles et al., 2002).

Data on phenethyl alcohol⁴ (syn. 2-phenylethan-1-ol) [FL-no: 02.019] and ethyl phenylacetate [FL-no: 09.784]⁵ are considered representative for some of the candidate substances (see footnotes). They have been tested for their ability to induce reverse mutations in various strains of *Salmonella typhimurium* (e.g. TA92, TA94, TA97, TA98, TA100, TA1535, TA1537 and TA1538) in the presence or absence of an exogenous metabolic activation system. None of the compounds was mutagenic in any of the tester strains when tested at concentrations up to 5000 microgram/plate.

There are some positive findings with two of the potential hydrolysis products of the two candidate acetals [FL-no: 06.078 and 06.080] *in vitro* and *in vivo*, ethanol and acetaldehyde. The genotoxicity of these two compounds is well known. However, they both do occur naturally in many foods in mg amount (apart from alcoholic beverages) (TNO, 2000) and, based on the MSDI approach, the estimated intakes of candidate flavouring substances which might be expected to be hydrolysed to the corresponding alcohols and aldehydes are much lower. Further, ethanol and acetaldehyde are endogenous. So, the daily *in vivo* formation of ethanol has been estimated to be 40-80 mg/kg body weight/day (JECFA, 1997a).

For the supporting substances, there are *in vitro* genotoxicity studies available from test systems other than bacterial, which were reported to be negative: no increase in sister chromatid exchange frequency was reported in human whole blood lymphocyte cultures exposed to phenethyl alcohol [FL-no: 02.019] for 72 hours; and ethyl phenylacetate [FL-no: 09.784] did not cause chromosomal aberrations in Chinese hamster fibroblasts when incubated for 48 hours.

From the available *in vitro* and *in vivo* mutagenicity data on the additional structurally related substance isoeugenyl phenylacetate there is no indication of a mutagenic activity: a negative result was reported in an Ames Test in various strains of *Salmonella typhimurium* (e.g. TA98, TA100, TA1535, TA1537 and TA1538) with and without metabolic activation and the substance was reported not to induce sex-linked recessive (lethal) mutations in *Drosophila melanogaster in vivo* (Wild et al., 1983).

⁴ supporting 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166]

⁵ supporting pentyl phenylacetate [FL-no: 09.761], menthyl phenylacetate [FL-no: 09.620], hex-2-enyl phenylacetate [FL-no: 09.400]

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

There are no genotoxicity studies available on 2-phenethyl acetals, neither from the group of candidate nor of supporting substances.

Conclusion on genotoxicity:

There are valid *in vitro* genotoxicity data available for one of the ten candidate substances [FL-no: 02.166] in this flavouring group evaluation. Valid *in vitro* and limited *in vivo* mutagenicity data are available for two of the supporting substances and on a further structurally related substance.

For the candidate substance 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166], the only available study gave no indication of a genotoxic potential *in vitro*, but disclosed a possible pro-oxidant activity of the substance.

From the various studies carried out with supporting substances, there is no indication of a genotoxic activity in bacterial mutation assays of the phenethyl alcohols, phenylacetic acids and related esters in this flavouring group evaluation.

Overall, the genotoxicity data available are not sufficient to evaluate the genotoxicity adequately, however, the data available on candidate and supporting substances do not give rise to concern with respect to genotoxicity of the ten candidate substances in this flavouring group evaluation.

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

9. Conclusions

The present Flavouring Group Evaluation deals with seven phenethyl alcohol derivatives (alcohol, esters and acetals), one phenethyl aldehyde derivative and two phenylacetates from chemical group 15.

Two of the ten candidate substances possess a chiral centre [FL-no: 06.080 and 09.686]. One candidate substance [FL-no: 09.620] possesses three chiral centres. These substances have been evaluated irrespective of their chirality.

Due to the presence and the position of a double bond, one of the candidate substances [FL-no: 09.620] can exist as geometrical isomers, but the geometrical isomeric form is given by name.

The ten flavouring substances are all classified into structural class I.

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

According to the default MSDI approach, the ten flavouring substances have intakes in Europe from 0.012 to 1.9 microgram/*capita*/day which are well below the threshold of concern for structural class I of 1800 microgram/person/day.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the ten candidate substances belonging to class I would result in a total intake of approximately 4.6 microgram/*capita*/day. This value is below the threshold of concern for structural class I of 1800 microgram/person/day. The combined intake of the candidate and supporting substances in Europe is approximately 2000 microgram/*capita*/day, which exceeds the threshold of concern for structural class I of 1800 microgram/person/day. However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

All ten substances are expected to be metabolised to innocuous substances at the estimated levels of use as flavouring substances.

Overall, the genotoxicity data available are not sufficient to evaluate the genotoxicity adequately. However, the data available on candidate and supporting substances do not give rise to concern with respect to genotoxicity of the ten candidate substances in this flavouring group evaluation. Consideration was given to ethanol and acetaldehyde, two potential hydrolysis products of the two candidate acetals [FL-no: 06.078 and 06.080]. Because of the natural occurrence in food and the endogenous formation in humans of considerably larger amounts of these compounds, their formation from hydrolysis of the acetals was not considered to be of safety concern with respect to genotoxicity at their estimated levels of intakes, based on the MSDI approach.

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 ten flavouring substances would not give rise to safety concerns at 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 3700 microgram/person/day for the ten flavouring substances from structural class I. Thus, the intakes were above the threshold of concern for structural class I of 1800 microgram/person/day except for one candidate substance [FL-no: 05.159]. The one substance [FL-no: 05.159], which has an mTAMDI intake below the threshold of concern for structural class I, is also expected to be metabolised to innocuous products.

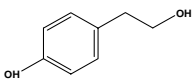
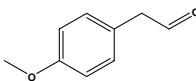
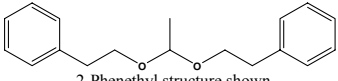
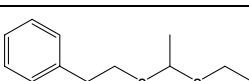
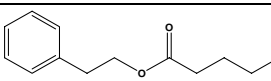
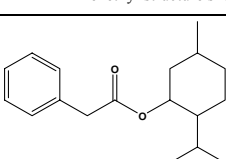
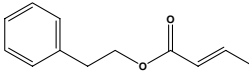
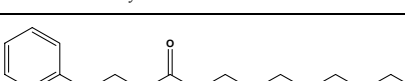
Thus, for nine of the ten flavouring substances [FL-no: 02.166, 06.078, 06.080, 09.201, 09.620, 09.684, 09.685, 09.686, and 09.761] considered in this opinion, the intakes estimated on the basis of the mTAMDI, exceed the relevant threshold for the structural class to which the flavouring substances have been assigned. Therefore, more reliable exposure data are required for these nine substances. 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 of the ten flavouring substances can be applied to the materials of commerce, it is necessary to consider the available specifications.

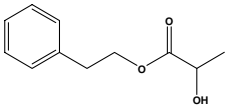
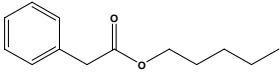
Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for all ten candidate substances, except that information on chirality is missing for two of the substances [FL-no: 09.620, 09.686] and information on the structure of the phenethyl moiety is lacking for four substances [FL-no: 06.078, 09.201, 09.684, and 09.686]. Thus, the final evaluation of the materials of commerce cannot be performed for five of the ten substances [FL-no: 06.078, 09.201, 09.620, 09.684, and 09.686], pending further information.

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 14

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 14								
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
02.166	2-(4-Hydroxyphenyl)ethan-1-ol		10226 501-94-0	Solid C ₈ H ₁₀ O ₂ 138.17	Slightly soluble 1 ml in 1 ml	310 91 MS 95 %	n.a. n.a.	
05.159	p-Methoxyphenylacetaldehyde		5703-26-4	Liquid C ₉ H ₁₀ O ₂ 150.18	Slightly soluble 1 ml in 1 ml	255 MS 95 %	1.531-1.537 1.093-1.099	
06.078	1,1-Diphenethoxyethane	 2-Phenethyl structure shown	122-71-4	Liquid C ₁₈ H ₂₂ O ₂ 270.37	Practically insoluble or insoluble 1 ml in 1 ml	198 (20 hPa) MS 95 %	1.533-1.539 1.028-1.034	1-Phenethyl or 2-phenethyl not indicated
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane		10049 2556-10-7	Liquid C ₁₂ H ₁₈ O ₂ 194.27	Practically insoluble or insoluble 1 ml in 1 ml	95 (5 hPa) MS 95 %	1.496-1.502 0.983-0.989	
09.201	Phenethyl valerate	 2-Phenethyl structure shown	673 7460-74-4	Liquid C ₁₃ H ₁₈ O ₂ 206.28	Practically insoluble or insoluble 1 ml in 1 ml	133 (13 hPa) MS 95 %	1.482-1.489 0.978-0.984	1-Phenethyl or 2-phenethyl not indicated
09.620	Menthyl phenylacetate 6)		26171-78-8	Liquid C ₁₈ H ₂₆ O ₂ 274.40	Practically insoluble or insoluble 1 ml in 1 ml	194 (13 hPa) NMR 95 %	1.500-1.506 0.999-1.005	CAS no reported refers to the (1R, 2S, 5R)-enantiomer
09.684	Phenethyl crotonate	 2-Phenethyl structure shown	10880 68141-20-8	Liquid C ₁₂ H ₁₄ O ₂ 190.24	Practically insoluble or insoluble 1 ml in 1 ml	271 MS 95 %	1.513-1.517 1.017-1.023	1-Phenethyl or 2-phenethyl not indicated
09.685	2-Phenethyl decanoate		10881 61810-55-7	Liquid C ₁₈ H ₂₈ O ₂ 276.42	Practically insoluble or insoluble 1 ml in 1 ml	206 (20 hPa) NMR 95 %	1.490-1.496 0.959-0.965	

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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
09.686	Phenethyl lactate 6)	 <p>2-Phenethyl structure shown</p>	155449-46-0	Solid C ₁₁ H ₁₄ O ₃ 194.23	Practically insoluble or insoluble 1 ml in 1 ml	355 55 MS 95 %	n.a. n.a.	(R) or (S) enantiomer not specified by CAS no reported 1-Phenethyl or 2-phenethyl not indicated
09.761	Pentyl phenylacetate		612 5137-52-0	Liquid C ₁₃ H ₁₈ O ₂ 206.28	Practically insoluble or insoluble 1 ml in 1 ml	268 MS 95 %	1.483-1.489 0.977-0.985	

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

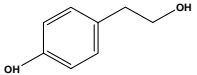
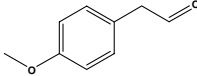
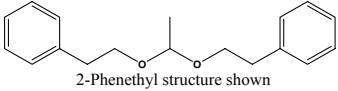
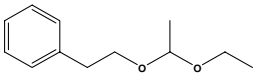
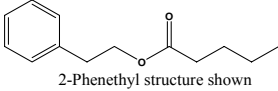
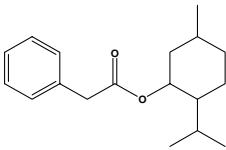
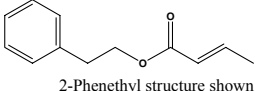
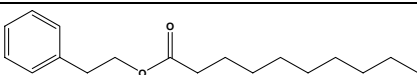
4) At 20°C, if not otherwise stated

5) At 25°C, if not otherwise stated

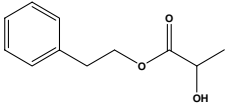
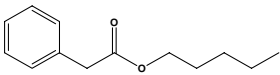
6) Stereoisomeric composition not specified

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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)							
FL-no	EU Register name	Structural formula	MSDI 1) ($\mu\text{g}/\text{capita}/\text{day}$)	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
02.166	2-(4-Hydroxyphenyl)ethan-1-ol		0.12	Class I A3: Intake below threshold	4)	6)	
05.159	p-Methoxyphenylacetaldehyde		0.037	Class I A3: Intake below threshold	4)	6)	
06.078	1,1-Diphenethoxyethane	 2-Phenethyl structure shown	0.012	Class I A3: Intake below threshold	4)	7)	
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane		0.012	Class I A3: Intake below threshold	4)	6)	
09.201	Phenethyl valerate	 2-Phenethyl structure shown	0.012	Class I A3: Intake below threshold	4)	7)	
09.620	Menthyl phenylacetate		1.5	Class I A3: Intake below threshold	4)	7)	
09.684	Phenethyl crotonate	 2-Phenethyl structure shown	0.73	Class I A3: Intake below threshold	4)	7)	
09.685	2-Phenethyl decanoate		0.037	Class I A3: Intake below threshold	4)	6)	

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	EU Register name	Structural formula	MSDI 1) ($\mu\text{g}/\text{capita}/\text{day}$)	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
09.686	Phenethyl lactate	 <p>2-Phenethyl structure shown</p>	0.24	Class I A3: Intake below threshold	4)	7)	
09.761	Pentyl phenylacetate		1.9	Class I A3: Intake below threshold	4)	6)	

1) $\text{MSDI} = \text{Amount added to food as flavour in (kg/year)} \times 10E9 / (0.1 \times \text{population in Europe (= } 375 \times 10E6) \times 0.6 \times 365) = \mu\text{g}/\text{capita}/\text{day}$

2) *Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 $\mu\text{g}/\text{person}/\text{day}$*

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.*

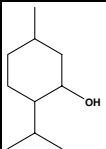
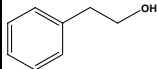
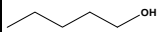


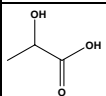
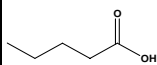
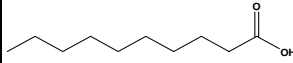
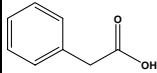
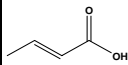
6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach)*

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.*

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

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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)	Structural class 4) Procedure path (JECFA) 5)	Comments
02.015	Menthol 427		No safety concern a) Category A b)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOEL exists	NOAEL: 380 mg/kg bw/d
02.019	2-Phenylethan-1-ol 987		No safety concern c) Category B b)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 d) No safety concern e) Category A b)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 d)	No evaluation	
05.001	Acetaldehyde 80		Category 1 d) No safety concern e) Category A b)	Class I A3: Intake below threshold	
08.004	Lactic acid 930		No safety concern f) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 d) No safety concern e) Category A b)	Class I A3: Intake below threshold	
08.011	Decanoic acid 105		Category 1 d) No safety concern e) Category A b)	Class I A3: Intake below threshold	
08.038	Phenylacetic acid 1007		No safety concern c) Category B b)	Class I A3: Intake below threshold	
08.072	But-2-enoic acid (cis and trans) 1371		No safety concern (JECFA, 2005)	No evaluation	JECFA evaluated (E)-isomer

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

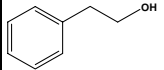
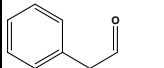
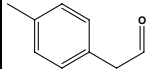
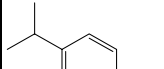
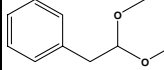
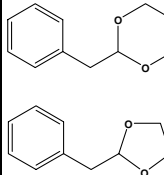
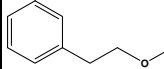
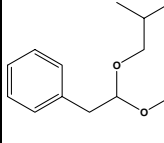
2) No safety concern at estimated levels of intake

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

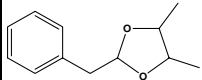
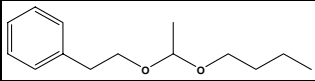
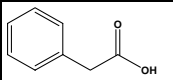
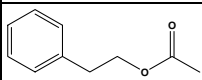
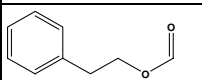
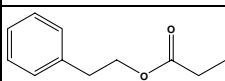
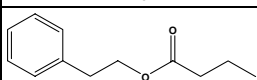
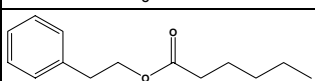
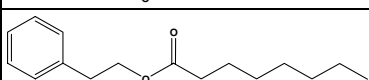
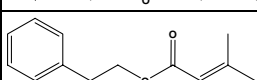
- 3) *Category A: Flavouring substance, which may be used in foodstuffs; Category B: Flavouring substance which can be used provisionally in foodstuffs*
- 4) *Threshold of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day*
- 5) *Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot*
 - a) *(JECFA, 2000a)*
 - b) *(CoE, 1992)*
 - c) *(JECFA, 2002c)*
 - d) *(SCF, 1995)*
 - e) *(JECFA, 1999b)*
 - f) *(JECFA, 2002b)*

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

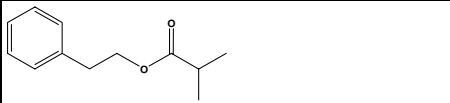
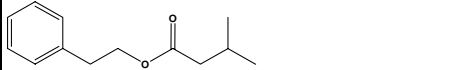
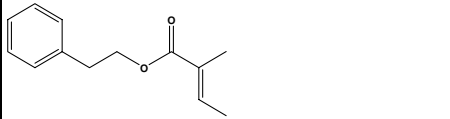
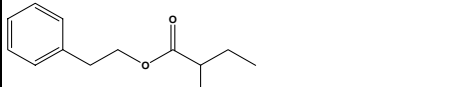
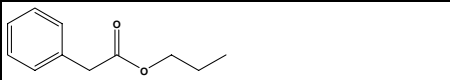
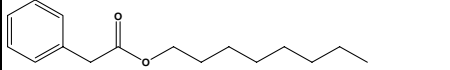
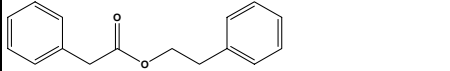
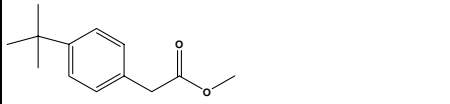
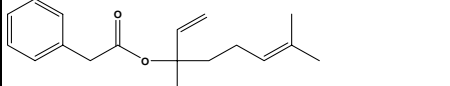
TABLE 3: SUPPORTING SUBSTANCES SUMMARY

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
02.019	2-Phenylethan-1-ol		2858 68 60-12-8	987 JECFA specification (JECFA, 2002d)	1200	No safety concern a) Category B b)	
05.030	Phenylacetaldehyde		2874 116 122-78-1	1002 JECFA specification (JECFA, 2002d)	37	No safety concern a) Category B b)	
05.042	p-Tolylacetaldehyde		3071 130 104-09-6	1023 JECFA specification (JECFA, 2002d)	5.5	No safety concern a) Category B b)	
05.044	p-Isopropyl phenylacetaldehyde		2954 132 4395-92-0	1024 JECFA specification (JECFA, 2002d)	0.061	No safety concern a) Category B b)	
06.006	1,1-Dimethoxy-2-phenylethane		2876 40 101-48-4	1003 JECFA specification (JECFA, 2002d)	17	No safety concern a) Category B b)	
06.007	Phenylacetaldehyde glyceryl acetal		2877 41 29895-73-6	1004 JECFA specification (JECFA, 2002d)	0.12	No safety concern a) Category B b)	
06.016	1-Phenylethoxy-1-propoxy ethane		2004 511 7493-57-4	1000 JECFA specification (JECFA, 2002d)	0.12	No safety concern a) Category B b)	
06.024	1,1-Di-isobutoxy-2-phenylethane		3384 595 68345-22-2	1006 JECFA specification (JECFA, 2002d)	27	No safety concern a) Category B b)	

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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 ($\mu\text{g}/\text{capita}/\text{day}$)	SCF status 2) JECFA status 3) CoE status 4)	Comments
06.027	4,5-Dimethyl-2-benzyl-1,3-dioxolan		2875 669 5468-06-4	1005 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to their real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992)
06.036	1-Butoxy-1-(2-phenylethoxy)ethane		3125 10007 64577-91-9	1001 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) -	
08.038	Phenylacetic acid		2878 672 103-82-2	1007 JECFA specification (JECFA, 2002d)	240	No safety concern a) Category B b)	
09.031	Phenethyl acetate		2857 221 103-45-7	989 JECFA specification (JECFA, 2002d)	89	No safety concern a) Category B b)	
09.083	Phenethyl formate		2864 350 104-62-1	988 JECFA specification (JECFA, 2002d)	2.1	No safety concern a) Category B b)	
09.137	Phenethyl propionate		2867 418 122-70-3	990 JECFA specification (JECFA, 2002d)	0.97	No safety concern a) Category B b)	
09.168	Phenethyl butyrate		2861 506 103-52-6	991 JECFA specification (JECFA, 2002d)	28	No safety concern a) Category B b)	
09.261	2-Phenethyl hexanoate		3221 10882 6290-37-5	995 JECFA specification (JECFA, 2002d)	12	No safety concern a) -	
09.262	Phenethyl octanoate		3222 10884 5457-70-5	996 JECFA specification (JECFA, 2002d)	23	No safety concern a) -	
09.407	2-Phenethyl 3-methylcrotonate		2869 246 42078-65-9	998 JECFA specification (JECFA, 2002d)	1.3	No safety concern a) Category B b)	

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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
09.427	Phenethyl isobutyrate		2862 302 103-48-0	992 JECFA specification (JECFA, 2002d)	19	No safety concern a) Category B b)	
09.466	Phenethyl isovalerate		2871 461 140-26-1	994 JECFA specification (JECFA, 2002d)	81	No safety concern a) Category B b)	
09.496	Phenethyl 2-methylcrotonate		2870 2186 55719-85-2	997 JECFA specification (JECFA, 2002d)	0.24	No safety concern a) Category B b)	
09.538	Phenethyl 2-methylbutyrate		3632 10883 24817-51-4	993 JECFA specification (JECFA, 2002d)	0.37	No safety concern a) -	
09.702	Propyl phenylacetate		2955 229 4606-15-9	1010 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
09.703	Octyl phenylacetate		2812 230 122-45-2	1017 JECFA specification (JECFA, 2002d)	0.0037	No safety concern a) Category B b)	
09.707	Phenethyl phenylacetate		2866 234 102-20-5	999 JECFA specification (JECFA, 2002d)	33	No safety concern a) Category A b)	
09.758	Methyl p-tert-butylphenylacetate		2690 577 3549-23-3	1025 JECFA specification (JECFA, 2002d)	17	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to their real use in foodstuffs and/or for which insufficient technological and/or toxicological information was available (CoE, 1992)
09.772	Linalyl phenylacetate		3501 655 7143-69-3	1019 JECFA specification (JECFA, 2002d)	0.073	No safety concern a) Category B b)	

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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 ($\mu\text{g}/\text{capita}/\text{day}$)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.783	Methyl phenylacetate		2733 2155 101-41-7	1008 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
09.784	Ethyl phenylacetate		2452 2156 101-97-3	1009 JECFA specification (JECFA, 2002d)	110	No safety concern a) Category B b)	
09.785	Citronellyl phenylacetate		2315 2157 139-70-8	1021 JECFA specification (JECFA, 2002d)	1.2	No safety concern a) Category B b)	
09.786	Isopropyl phenylacetate		2956 2158 4861-85-2	1011 JECFA specification (JECFA, 2002d)	0.061	No safety concern a) Category B b)	
09.787	Butyl phenylacetate		2209 2159 122-43-0	1012 JECFA specification (JECFA, 2002d)	2.4	No safety concern a) Category B b)	
09.788	Isobutyl phenylacetate		2210 2160 102-13-6	1013 JECFA specification (JECFA, 2002d)	18	No safety concern a) Category B b)	
09.789	3-Methylbutyl phenylacetate		2081 2161 102-19-2	1014 JECFA specification (JECFA, 2002d)	28	No safety concern a) Category B b)	
09.791	Rhodinyl phenylacetate		2985 2163 10486-14-3	1018 JECFA specification (JECFA, 2002d)	0.0012	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to their real use in foodstuffs and/or for which insufficient technological and/or toxicological information was available (CoE, 1992)
09.804	Hexyl phenylacetate		3457 10694 5421-17-0	1015 JECFA specification (JECFA, 2002d)	6.9	No safety concern a) -	
09.805	Hex-3(cis)-enyl phenylacetate		3633 10682 42436-07-7	1016 JECFA specification (JECFA, 2002d)	0.73	No safety concern a) -	JECFA did not specify isomer

1) 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) = $\mu\text{g}/\text{capita}/\text{day}$

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

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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, 2002c)*

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) that are not considered to present a safety concern have been specified.

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 products⁶ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous⁷ (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.

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.

⁶ "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).

⁷ "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).

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

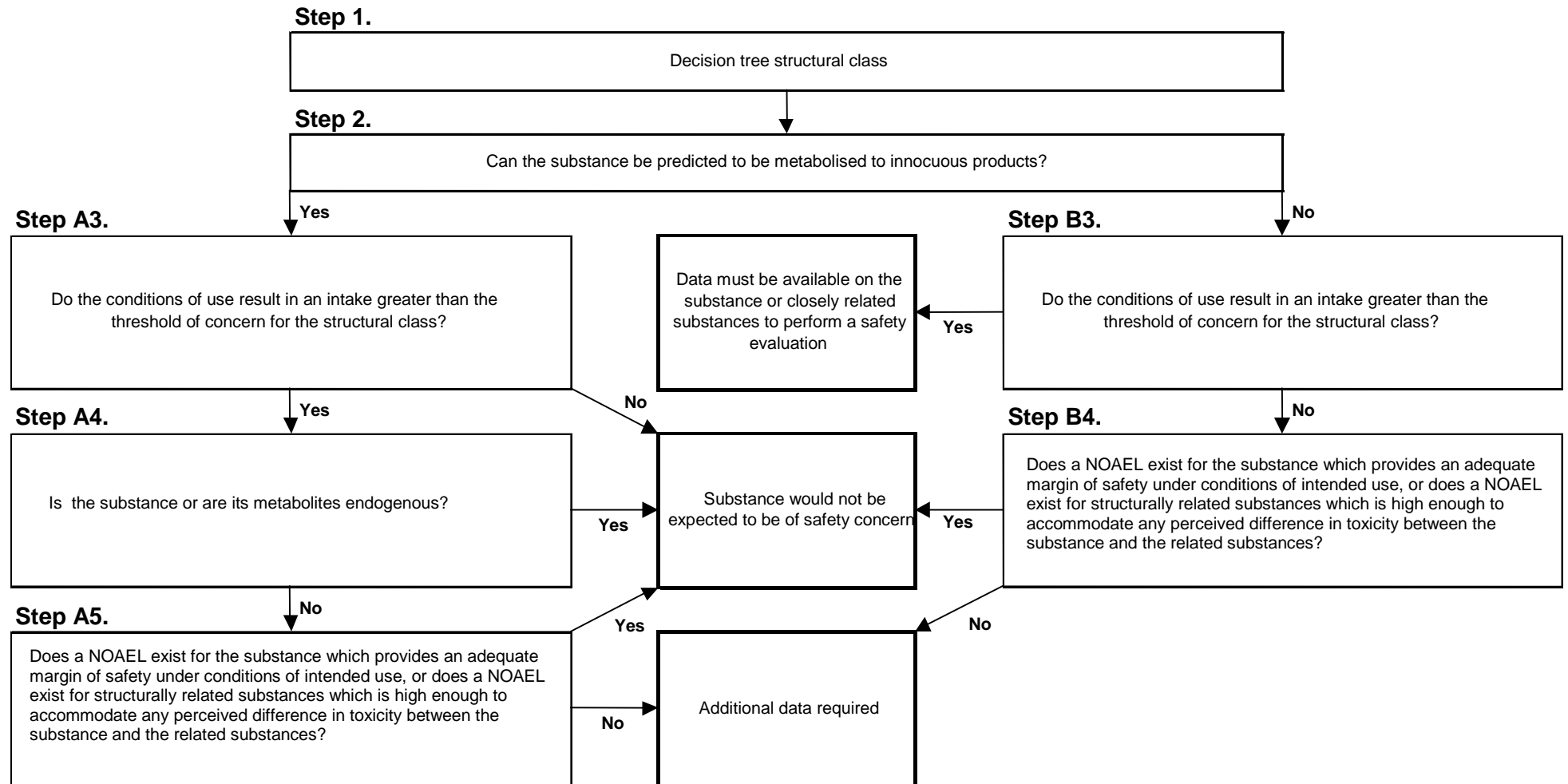


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

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).

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 ten candidate substances in the present flavouring group (Table II.1.2).

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
02.166	7	5	10	7	-	10	5	10	2	2	-	-	5	10	-	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	-	-	100	25
05.159	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	-	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	-	25	10
06.078	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
06.080	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
09.201	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
09.620	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
09.684	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
09.685	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
09.686	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
09.761	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	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.

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionary	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

Key	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
		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		

Flavouring Group Evaluation 14

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

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

Table II.2.3 mTAMDI ($\mu\text{g}/\text{person}/\text{day}$) for substances allocated to structural class I. (Threshold of concern for structural class I: 1800 $\mu\text{g}/\text{person}/\text{day}$)					
FL-no	EU Register name	MSDI ($\mu\text{g}/\text{capita}/\text{day}$)	mTAMDI ($\mu\text{g}/\text{person}/\text{day}$)	Structural class	Threshold of concern ($\mu\text{g}/\text{person}/\text{day}$)
02.166	2-(4-Hydroxyphenyl)ethan-1-ol	0.12	2100	Class I	1800
05.159	p-Methoxyphenylacetaldehyde	0.037	1600	Class I	1800
06.078	1,1-Diphenethoxyethane	0.012	3700	Class I	1800
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane	0.012	3700	Class I	1800
09.201	Phenethyl valerate	0.012	3700	Class I	1800
09.620	Menthyl phenylacetate	1.5	3700	Class I	1800
09.684	Phenethyl crotonate	0.73	3700	Class I	1800
09.685	2-Phenethyl decanoate	0.037	3700	Class I	1800
09.686	Phenethyl lactate	0.24	3700	Class I	1800
09.761	Pentyl phenylacetate	1.9	3700	Class I	1800

ANNEX III: METABOLISM

III.1. Absorption, distribution, and excretion

Flavouring Group Evaluation 14 includes ten substances structurally related to phenethyl alcohol, phenethyl aldehyde and phenylacetic acid. Six of the candidate substances are esters. The chemical group also contains two acetals in which the phenyl groups are localised at the alcohol moieties. In addition an alcohol and aldehyde are contained of which the first contains a hydroxyphenyl group and the second contains a methoxyphenyl group.

One candidate substance, 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166], is reported to be rapidly absorbed in humans followed by conjugation and excretion in the urine (Vissers et al., 2002). For the rest of the candidate substances no data on absorption and distribution are available.

III.2. Biotransformation

The first step in the biotransformation of the esters and acetals is hydrolysis.

III.2.1 Ester hydrolysis

The six esters included in this monograph are expected to be hydrolysed to carboxylic acids and alcohols by carboxylesterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980). In mammals these enzymes occur within the body in most tissues including the gut lumen and intestinal wall, but predominate in the hepatocytes (Heymann, 1980). The wide range of tissue distribution and the multiplicity of the esterases generally give rise to rapid hydrolysis of esters *in vivo*.

No hydrolysis studies on the candidate esters are available. Hydrolysis of several supporting substances like methyl phenylacetate, ethyl phenylacetate, phenylethyl acetate, isoamyl phenylacetate and isopropyl phenylacetate was examined in gastrointestinal juice. The data obtained for hydrolysis of supporting substances by pancreatic enzymes (50-100% hydrolysis after 2 hours of incubation of 37°C) indicate the potentials of these enzymes to hydrolyse these candidate substances (Grundschober, 1977; Longland et al., 1977). The results showed that the *in vitro* hydrolysis rates of the supporting substances were relatively slow by artificial gastric juice, but the substances were easily hydrolysed by pancreatic enzymes, with which 50-100 % hydrolysis of the supporting substances was observed after two hours of incubation at 37°C. Based on the hydrolysis studies of the supporting esters, the candidate esters phenethyl lactate, phenethyl valerate, 2-phenethyl decanoate, phenethyl crotonate, pentyl phenylacetate and menthyl phenylacetate [FL-no: 09.686, 09.201, 09.685, 09.684, 09.761 and 09.620] are predicted to undergo hydrolysis to phenethyl alcohol, phenylacetic acid, and simple alcohols and acids.

III.2.2 Hydrolysis of acetals

Acetals are formed from reaction between alcohols and aldehydes/ketones in the presence of anhydrous acids. With large aldehydes and ketones, the equilibrium constant for acetal formation is generally unfavourable. Acetals are stable in basic conditions, but are hydrolysed back to carbonyl compounds under acidic conditions (Streitwieser et al, 1992; Carey, 1992). However, there is limited knowledge of the rate of hydrolysis and the impact of chemical structure on the hydrolysis rate.

No hydrolysis data have been found for the two candidate acetals 1-ethoxy-1-(2-phenylethoxy)ethane [FL-no: 06.080] and 1,1-diphenethoxyethane [FL-no:06.078]. JECFA evaluated two supporting substances in 2003, 1-phenylethoxy-1-propoxy ethane [FL-no: 06.016] and 1-butoxy-1-(2-phenylethoxy)ethane [FL-no: 06.036] (JECFA, 2003a). Like the candidate acetal, the supporting acetals have phenyl groups at the alcohol parts of the substances, but only one methyl group at the central C-carbon on the aldehyde part of the substance. JECFA based the evaluation of 1-phenylethoxy-1-propoxy ethane [FL-no: 06.016] and 1-butoxy-1-(2-phenylethoxy)ethane [FL-no: 06.036] on the hydrolysis of 2-phenylpropanal dimethyl acetal and benzaldehyde propylene glycol acetal (Morgareidge, 1962a) (Figure III.1). 2-Phenylpropanal dimethyl acetal and benzaldehyde propylene glycol acetal were hydrolysed by 97% after 1 h and 53% after 5 h, respectively in simulated gastric juice. Benzaldehyde propylene glycol acetal was in addition shown to be hydrolysed by 99% in 0.1M HCl under reflux after 1 h (Morgareidge, 1962a).

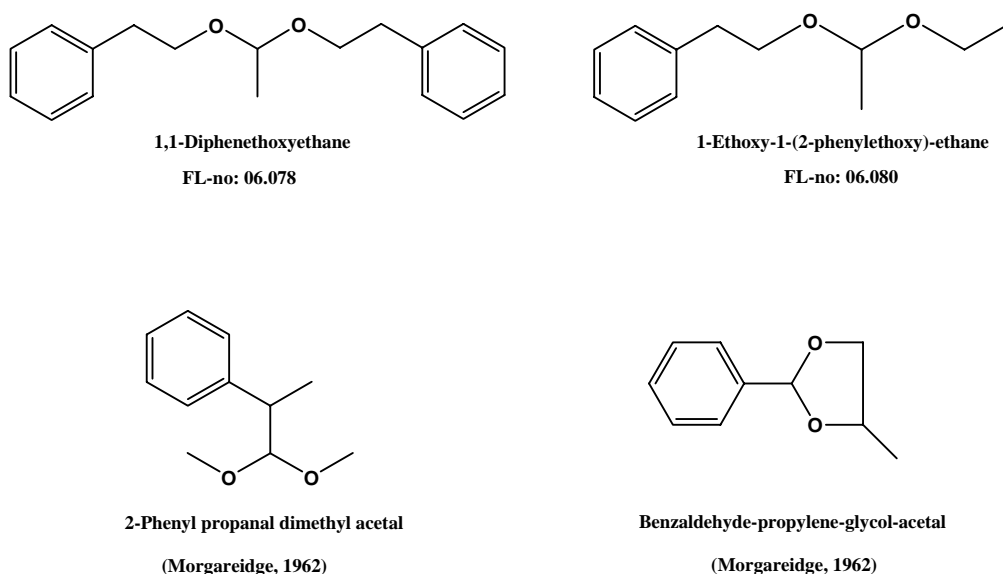


Figure III.1. Chemical structure of candidate substances and related acetals.

However, there are major differences in the chemical structure between 2-phenylpropanal dimethyl acetal and benzaldehyde propylene glycol acetal and the candidate substances [FL-no: 06.080, 06.078] and the supporting substances [FL-no: 06.016, 06.036], since 2-phenylpropanal dimethyl acetal and benzaldehyde propylene glycol acetal have their phenyl group at the central C-atom in the aldehyd part of the substance, while the two candidate acetals have the aromatic ring in the alcohol group(s). In addition the author questioned the chemical identity of the sample with benzaldehyde propylene glycol acetal, making the hydrolysis data in this article rather inconclusive. The hydrolysis data given by Morgareidge (1962) are not suitable to predict the hydrolysis rate of the candidate acetals or the supporting acetals. In the few hydrolysis experiments done on acetals, an increase in hydrolysis rate has been found with a higher degree of substitution at the central C-atom (Kreevoy & Taft, 1955a; Pchelintsev et al., 1988; Deslongchamps et al., 2000). Changing from one methyl group to one phenyl group at the central C-atom would increase the hydrolysis rate about 30 times (Kreevoy & Taft, 1955a; Pchelintsev et al., 1988). See Annex III. in FGE.03 for a more comprehensive evaluation of acetal hydrolysis.

From available data on acetals with differing chemical structures it is clear that the rates of both acid hydrolysis and enzymatic hydrolysis will vary depending on the structure, and that hydrolysis

sometimes may be slow and incomplete. Data submitted show that the rate of hydrolysis may vary considerably even within groups of closely related substances with simple structures. The rate of hydrolysis may also depend on the solubility of the substance in aqueous media. However, in vitro experiments using simulated gastric fluid revealed the rates of hydrolysis of acetals to be dependent on the structures of the aldehyde and alcohol moieties. Acetals derived from short (<C8) chain saturated aldehydes, were hydrolyzed almost instantly (Engel, 2003).

It is therefore anticipated that the two candidate substances 1-ethoxy-1-(2-phenylethoxy)ethane [FL-no: 06.080] and 1,1-diphenethoxyethane [FL-no:06.078] would undergo hydrolysis in the gastric environment.

III.3. Further metabolism of candidate substances and their hydrolysis products

III.3.1 Metabolism of 2-(4-hydroxyphenyl)ethan-1-ol [FL-no: 02.166] (tyrosol)

Tyrosol is naturally occurring in olive oil, and its absorption and excretion have recently been studied both in rats and humans (Vissers et al., 2002; Tuck et al., 2001; Bonanome et al., 2000; Miró-Casas et al., 2001a; Miró-Casas et al., 2001b; Visioli et al., 2000). When 100 mg olive oil phenols were administered orally to humans, most of the phenols was absorbed in the intestine, shown by low excretion of olive oil phenols into ileostomy effluent and 55-69% recovery of phenols in the urine (Vissers et al., 2002). Increased tyrosol content in the lipid fraction of human blood after orally administered olive oil supports the view that tyrosol is readily absorbed from the gastrointestinal tract (Bonanome et al., 2000). One of the main problems when estimating the bioavailability of phenolic compounds from olive oil in humans is the estimation of the dosage administered, since these substances can be present in multiple forms in food. The extraction procedures used when estimating the tyrosol content in olive oil also affects the dosage estimates (Miró-Casas et al., 2001a). However, more than 85% of the amount of tyrosol excreted in the urine as glucuronide (Miró-Casas et al., 2001a; Visioli et al., 2000), with reported recovery in human urine from 12 to 52% of the total estimated dosage after 24 hours (Miró-Casas et al., 2001a; Miró-Casas et al., 2001b). Only 6-11% of the total tyrosol excreted was in free form (Miró-Casas et al., 2001b). A bioavailability study in rat where radioactively labelled tyrosol in olive oil or aqueous solution was given by gavage measured a 99% and 75% bioavailability after 24 hours from olive oil and aqueous solution, respectively (Tuck et al., 2001). Altogether, it is reasonable to believe that most of the tyrosol is readily absorbed and excreted as glucuronide in the urine.

III.3.2 Metabolism of phenethyl alcohol, phenyl acetaldehyde and phenyl acetic acid

The extent to which an alcohol is oxidized enzymatically depends upon its structure. For example, alcohol dehydrogenase was reported to have a lower K_m for 3-phenylpropanol than for propanol, but in this case the V_{max} was also reduced by a factor of 2.5 (Pietruszko et al., 1973).

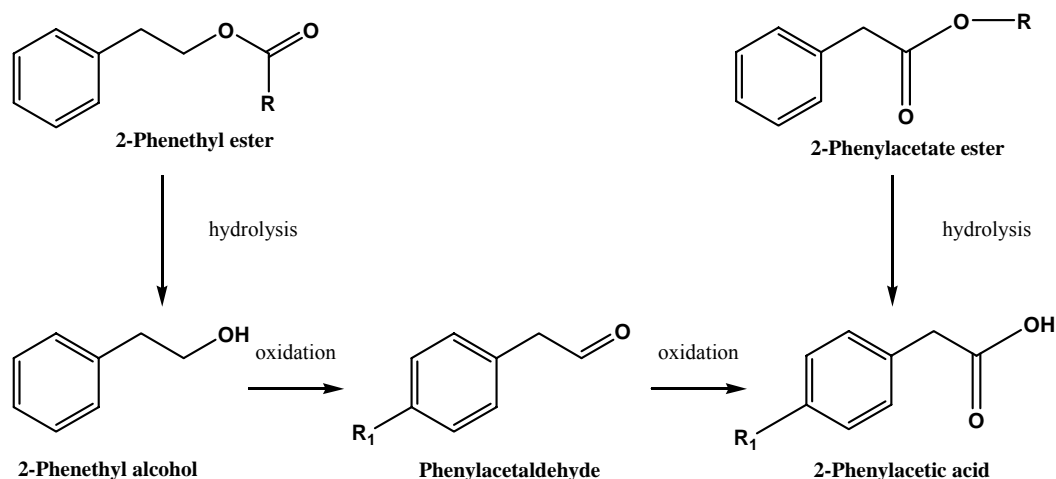


Figure III.2. Metabolism of 2-phenethyl alcohol, 2-phenylacetaldehyde and 2-phenylacetic acid. $R_1 = H$ - or CH_3 -O

Phenethyl alcohol and phenylacetic acid are two of the major hydrolysis products from the ester and acetal hydrolysis (Figure III.2). No metabolism data is found for the candidate substance *p*-methoxyphenylacetaldehyde [FL-no: 05.159]. Phenethyl alcohol is first oxidised to phenylacetaldehyde, and thereafter it is further oxidised to phenylacetic acid, with a K_m of 29 nM and 180 μ M for cytosolic and mitochondrial liver aldehyde dehydrogenase respectively (Klyosov, 1996; Martini & Murray, 1996).

In humans, 26% of a 4000 mg oral dose of phenethyl alcohol is excreted in urine as phenylacetylglutamine within 24 hours (Thierfelder & Schempp, 1917). In two adult male humans, an average of 91% and 7% of a 1 mg/kg bw oral dose of [carboxy-¹⁴C]phenylacetic acid is excreted within 24 hours as glutamine and taurine conjugates, respectively. Unlike most other animals, only a trace of the glycine conjugate has been detected in humans (James et al., 1972). The distribution and type of conjugation is relatively unaffected by continued ingestion of phenylacetic acid. After being fed thirty-four 1000 to 10000 mg doses of the acid over a 97-day period, one human excreted greater than 90% of the administered dose as the phenylacetylglutamine conjugate (Ambrose et al., 1933). The capacity for glutamine conjugation has been studied in three volunteers and three patients exhibiting phenylketonuria (James & Smith, 1973). Each subject was given a single 80 mg dose of [carboxy-¹⁴C]-phenylacetic acid. The average excretion of phenylacetylglutamine (measured as mmol/g creatinine) by phenylketonurics was approximately five times that of the control subjects indicating that the glutamine conjugation mechanism is able to cope with large amounts of phenylacetic acid produced by phenylketonurics. The mechanism for conjugation of glutamine with phenylacetic acid involves formation of a phenylacetic acid coenzyme-A (CoA) intermediate (Moldave & Meister, 1957). One-hour perfusion of human kidney or incubation of human liver homogenate with [¹⁴C]-glutamine and phenylacetyl-CoA results in the formation of the radioactive conjugate in the respective yields of 5 and 13%.

Similar to humans, monkeys conjugate phenylacetic acid with glutamine and to a lesser extent, taurine. However, significant quantities of acid (1 – 44%) are excreted in the free form. In carnivores (e.g. dog, cat, ferret), glycine conjugation predominates with no detectable amounts of glutamine conjugation. Likewise in rodents and rabbits, phenylacetic acid is excreted primarily as the glycine conjugate. Unconjugated phenylacetic acid and minute amounts of taurine conjugates are also excreted.

In rabbits, 42% and 5% of a single 300 mg/kg bw oral dose of phenethyl alcohol is excreted in the urine as glycine and glucuronic acid conjugates of phenylacetic acid, respectively, within 24 hours.

The ether soluble acid extracted from the 24-hour urine accounted for 61% of the dose (Bray et al., 1958).

Greater than approximately 94% of an 80 mg/kg dose of phenylacetic acid given by intraperitoneal injection is excreted as the glycine conjugate in rats (James et al., 1972). In rats, endogenous levels of unconjugated phenylacetic acid may occur at dose levels at which glycine conjugation is capacity-limited, presumably by the supply of endogenous glycine (Gregus et al., 1993). Only small amounts of the glycine conjugate enter the bile. Less than 10% of a 100 mg/kg bw oral dose of phenylacetic acid was collected from the bile of rats over four hours (Koss & Lamprecht, 1968). Significant levels of free phenylacetic acid have been observed at high dose levels (Teuchy et al., 1971; James et al., 1972).

III.4 Conclusion

The six esters [FL-no: 09.686, 09.201, 09.685, 09.684, 09.761, and 09.620] included in this Flavouring Group Evaluation are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the evaluation of supporting substances (Grundschober, 1977; Longland et al., 1977).

It is anticipated that the two candidate acetals 1-ethoxy-1-(2-phenylethoxy)ethane [FL-no: 06.080] and 1,1-diphenethoxyethane [FL-no:06.078] would undergo hydrolysis in the gastric environment. Experiments using simulated gastric fluid revealed the rates of hydrolysis of acetals to be dependent on the structures of the aldehyde and alcohol moieties. Acetals derived from short (<C8) chain saturated aldehydes, were hydrolyzed almost instantly.

The carboxylic acids resulting from hydrolysis of candidate chemicals: lactic acid, valeric acid, decanoic acid, and crotonic acid are expected to be metabolised via common pathways, including beta-oxidation and citric acid cycle. From the hydrolysis of candidate acetals acetaldehyde is obtained, which is expected to be oxidised to acetic acid by aldehyde dehydrogenase. Acetic acid will follow the same metabolism as the carboxylic acids above.

The metabolic elimination of alcohols in experimental animals and man occurs primarily by two pathways: (1) oxidation to aldehyde and subsequently to the corresponding carboxylic acid, and (2) conjugation of the alcohol with glucuronic acid.

The candidate alcohol, [FL-no: 02.166], is absorbed in the intestine of humans, and mainly excreted in the urine conjugated with glucuronic acid. The candidate aldehyde, *p*-methoxyphenylacetaldehyde [FL-no: 05.159], is oxidized to the corresponding carboxylic acid and conjugated to amino acids like glutamine and glycine before excretion in the urine.

In addition, menthol is released after hydrolysis of menthyl phenylacetate [FL-no: 09.620]. Menthol is mainly conjugated with glucuronic acid and excreted by the bile in rats (Yamaguchi et al., 1994). Oxidation of the alkyl ring substituents may occur before conjugation with glucuronic acid. Low levels of oxidation products were found in the urine, but no unchanged menthol was detected in the urine, faeces or bile (Yamaguchi et al., 1994).

Phenethyl alcohol and phenylacetic acid are major hydrolysis products. Phenylacetic acid glucuronide is present in human urine, and is an endogenous end-product of phenylalanine metabolism (Seakins, 1971). The types of conjugates formed from phenylacetic acid are both dose-dependent and species-specific. The major metabolic options available to phenylacetic acid are conjugation with glucuronic acid, glycine, taurine or glutamine, or elimination as the free acid. In humans, phenylacetic acid is mainly excreted conjugated with glutamine.

In summary, the ten candidate substances are expected to be metabolised to innocuous products at the estimated levels of intake.

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

ANNEX IV: TOXICITY

Oral acute toxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 15, and for 24 supporting substances evaluated by JECFA at the 59th meeting. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Table IV.1: ACUTE TOXICITY						
Chemical Name	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Phenethyl alcohol [02.019])	Mouse	NR	Oral	800 – 1500	(Fassett, 1963)	
	Guinea pig	NR	Oral	400 – 800	(Fassett, 1963)	
	Rat	NR	Oral	2234 ¹	(Zeller & Hoffmann, 1974)	
	Rat	NR	Oral	1800	(Rumyantsev et al., 1987)	
	Rat	M	Oral	1900 ²	(Moreno, 1982e)	
	Rat	M	Oral	2100 ³	(Moreno, 1982f)	
	Rat	M, F	Gavage	1790	(Jenner et al., 1964)	
	Rat, Mouse and Guinea pig	M, F	Gavage	2540	(Zaitsev & Rakhmanina, 1974)	
	Rat	M, F	Gavage	1609	(Mallory et al., 1982)	
	Rat	M, F	Oral	2509 ¹	(Carpenter et al., 1974)	
(Phenethyl formate [09.083])	Rat	F	Gavage	3100 ⁴	(Grote & Woods, 1955)	
(Phenethyl acetate [09.031])	Rat	M, F	Gavage	3220	(Levenstein, 1972c)	
	Rat	NR	Oral	> 5000	(Moreno, 1973e)	
	Rat, Mouse and Guinea pig	M, F	Gavage	5200	(Rumyantsev et al., 1987)	
(Phenethyl propionate [09.137])	Rat, Mouse and Guinea pig	M, F	Gavage	3670	(Zaitsev & Rakhmanina, 1974)	
	Rat	NR	Oral	5200	(Rumyantsev et al., 1987)	
(Phenethyl propionate [09.137])	Rat	M, F	Gavage	3980 ⁵	(Beroza et al., 1975)	
(Phenethyl butyrate [09.178])	Rat	NR	Oral	4000	(Moreno, 1973f)	
(Phenethyl butyrate [09.178])	Rat	NR	Oral	4600	(Levenstein, 1974d)	
(Phenethyl isobutyrate [09.427])	Rat	M, F	Gavage	5137 ⁶	(Shelanski & Moldovan, 1971b)	
(Phenethyl 2-methylbutyrate [09.538])	Rat	NR	Oral	> 5000	(Moreno, 1982g)	
(Phenethyl tiglate [09.496])	Rat	NR	Oral	> 5000	(Levenstein, 1974e)	
(Phenethyl phenylacetate [09.707])	Rat, Mouse and Guinea pig	M, F	Gavage	3190	(Zaitsev & Rakhmanina, 1974)	
	Rat	M, F	Gavage	15390	(Jenner et al., 1964)	
1-Ethoxy-1-(2-phenylethoxy)ethane [06.080]	Rat	NR	Oral	> 5000	(Moreno, 1974f)	
(Acetaldehyde phenethyl propyl acetal [06.016])	Rat	NR	Oral	> 5000	(Moreno, 1979c)	
(Phenylacetaldehyde [05.030])	Rat	NR	Oral	1550	(Moreno, 1977m)	

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

Table IV.1: ACUTE TOXICITY						
Chemical Name	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
	Rat, Mouse and Guinea pig	M, F	Gavage	3890	(Zaitsev & Rakhmanina, 1974)	
(Phenylacetaldehyde dimethyl acetal [06.006])	Rat	M, F	Gavage	< 5000	(Shelanski & Moldovan, 1971c)	
(Phenylacetaldehyde glyceryl acetal [06.007])	Rat	M	Oral	1720	(Moreno, 1972c)	
	Rat	M	Oral	< 5000	(Moreno, 1972d)	
(Phenylacetic acid [08.038])	Rat	NR	Oral	> 5000	(Keating, 1972c)	
	Rat, Mouse and Guinea pig	M, F	Gavage	2250	(Zaitsev & Rakhmanina, 1974)	
(Methyl phenylacetate [09.783])	Rat	NR	Oral	2550	(Moreno, 1974g)	
(Ethyl phenylacetate [09.784])	Rat	NR	Oral	3300	(Moreno, 1973g)	
(Butyl phenylacetate [09.787])	Rat	NR	Oral	> 5000	(Moreno, 1980i)	
(Isobutyl phenylacetate [09.788])	Rat	NR	Oral	> 5000	(Moreno, 1973h)	
(Isoamyl phenylacetate [09.789])	Rat	NR	Oral	> 5000	(Moreno, 1976m)	
(3-Hexenyl phenylacetate [09.805])	Rat	NR	Oral	> 5000	(Moreno, 1976n)	
(Rhodinyl phenylacetate [09.791])	Rat	NR	Oral	> 5000	(Moreno, 1977n)	
(Linalyl phenylacetate [09.772])	Rat	NR	Oral	> 5000	(Moreno, 1974h)	
	Mouse	M, F	Oral	15488	(Colaiani, 1967)	
(Citronellyl phenylacetate [09.785])	Rat	NR	Oral	> 5000	(Moreno, 1977o)	
(Menthyl phenylacetate [09.620])	Rat	NR	Oral	> 5000	(Moreno, 1982d)	
(p-Tolylacetaldehyde [05.042])	Mouse	NR	Oral	> 5000	(Levenstein, 1975d)	

M = Male; F = Female.

NR = Not reported.

1 Value based on specific gravity = 1.02.

2 Test substance administered as 5% musk ambrette in phenethyl alcohol.

3 Test substance administered as 20% musk ambrette in phenethyl alcohol.

4 Test substance administered as beta-phenethyl alcohol.

5 Test substance administered as phenethyl propionate and eugenol, in a 7:3 ratio.

6 Value based on specific gravity = 0.98790.

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

Subacute / subchronic / chronic / Carcinogenic toxicity data are available for none of the candidate substances of the present flavouring group evaluation from chemical group 15 but for four supporting substances evaluated by JECFA at the 59th 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	Species; Sex No./Group	Route	Dose levels	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
(Phenethyl alcohol [02.019])	Rat; M, F 40	Drinking water	0 and 12% in a mixture in tap water	56 weeks	120 (0.12%) ¹	(Johannsen & Purchase, 1969)	Only a mixture was tested. Mixture gave no effects in liver, kidney, spleen, lung or hart. Limited information. The mixture is of different compounds
	Rat; M 12	Gavage	50.8 mg/kg bw	4 months	50.8 ¹	(Zaitsev & Rakhmanina, 1974)	Enzymatic activity in blood and liver was measured. Slightly stress on the liver. No histopathology. Apparently no control group. Study of low validity
(Phenethyl acetate [09.031])	Rat; M 12	Gavage	73.4 mg/kg bw	4 months	73.4 ¹	(Zaitsev & Rakhmanina, 1974)	Enzymatic activity in blood and liver was measured. Slightly stress on the liver. No histopathology. Apparently no control group. Study of low validity
(Phenethyl phenylacetate [09.707])	Rat; M, F 20	Diet	0, 1000, 2500 and 10000 ppm	17 weeks	500 ¹	(Hagan et al., 1967)	Only weight and macroscopic evaluation of tissues.
(<i>p</i> -Isopropylphenylacetaldehyde [05.044])	Rat; M, F 20 – 32	Diet	0, 16.98 (M) and 18.77 (F) mg/kg bw/day	90 days	M: 16.98 ¹ F: 18.77 ¹	(Posternak et al., 1969)	Haematological examination. Histological examination and weight of liver and kidney. No significant effect.

M = Male; F = Female. NR = Not reported.

¹ This study was performed at either a single dose level or multiple dose levels that produced no adverse effects.

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

Developmental and reproductive toxicity data are available for none of the candidate substances of the present flavouring group evaluation from chemical group 15 but for two supporting substance evaluated by JECFA at the 59th meeting. The supporting substances are listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Table IV.3: Developmental and Reproductive Toxicity Studies							
Chemical Name	Study type Durations	Species/Sex No/group	Route	Dose levels	NOAEL (mg/kg/day) Including information on possible maternal toxicity	Reference	Comments
(Phenethyl alcohol [02.019])	Developmental Toxicity: Gestation Days 6 - 15	Rat; F 5 – 7	Gavage	0, 4.3, 43 and 432 mg/kg bw	Maternal: 43 Foetal: < 4.3 ¹	(Mankes et al., 1983) (Mankes et al., 1984) (Mankes et al., 1985)	Foetal effects at 4.3 mg/kg not increased at higher doses.
	Developmental Toxicity: Gestation Days 6 - 15	Rat; F NR	Diet	0, 1000, 3000 and 10000 ppm	Maternal: 150 Foetal: 150	(Burdock et al., 1987)	No conc. related teratogenicity. Highest conc. gave weight loss of the dams. No detailed report of the results.
	Developmental Toxicity: Gestation Days 6 - 15	Rat; F 26 – 27	Diet	0, 1000, 3000 and 10000 ppm	Maternal: 266 Foetal: 266	(Bottomley et al., 1987)	Good quality study. Marginal delay in the ossification process of the litter at 10000 ppm. Probably related to slight weight loss of the dams. No teratogenic effects
	Developmental Toxicity: Gestation Days 6 - 15	Rat; F 21 – 34	Dermal	0, 0.14, 0.43 and 1.40 ml/kg bw	Maternal: 438 Foetal: 143	(Palmer et al., 1986)	GLP study. Clear maternal and embryo-foetal toxicity at the highest dosage. Some morphological changes in the foetuses at the middle dosage.
	Developmental Toxicity: Gestation Days 4 or 10 - 12	Rat; F 15 – 20	Oral	NR	Maternal: NR Foetal: < 508 ²	(Maganova & Zaitsev, 1973)	Russian. Only abstract in English.
(Phenylacetic acid [08.038])	Developmental Toxicity: Gestation Days 4 or 10 – 12	Rat; F 15 – 20	Oral	NR	Maternal: NR Foetal: < 450 ^{2,3}	(Maganova & Zaitsev, 1973)	Russian. Only abstract in English.
	Developmental Toxicity: Gestation Days 1 - 20 or 22	Rat; F 14 – 15	Oral	NR	Maternal: NR Foetal: 5 ³	(Zaitsev & Maganova, 1975)	Russian. Only abstract in English.
	Preliminary Developmental Toxicity: Gestation Days 6 – 15	Rat;F 24	Gavage	0, 100, 300 and 1000 mg/kg bw	Maternal: 300 Foetal: 1000	(Ridgway, 1986)	GLP study. No evidence for teratogenic effect.

M = Male; F = Female. NR=Not reported.

¹Results of these screening studies are inconsistent (most effects were not dose-related) and contradicted by results of more robust studies (Burdock et al., 1987; Bottomley et al., 1987; Palmer et al., 1986).

²Single dose level study, therefore the NOEL could not be determined. Results are contradicted by a definitive study in which phenethyl alcohol administered orally to pregnant rats at high doses during critical periods of embryogenesis did not cause any visible anomalies in embryonic development (Bottomley et al., 1987; Burdock et al., 1987).

³Results are contradicted by a definitive study in which phenethyl alcohol administered orally to pregnant rats at high doses during critical periods of embryogenesis did not cause any visible anomalies in embryonic development (Bottomley et al., 1987; Burdock et al., 1987).

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

In vitro mutagenicity/genotoxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 15 and for seven supporting substances evaluated by JECFA at the 59th meeting, and one structurally related substance evaluated at the 61st JECFA meeting. The supporting substances are listed in brackets.

TABLE IV.4: GENOTOXICITY (IN VITRO)

Table IV.4: GENOTOXICITY (<i>in vitro</i>)						
Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
(Phenethyl alcohol [02.019])	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate (366 µg/plate) ⁵	Negative ¹	(Florin et al., 1980)	Published non-GLP study. Limited report of study details. No results reported. Validity of the study cannot be evaluated.
	Ames reverse mutation assay	<i>S. typhimurium</i> TA100, TA1535, TA1538	0 - 99.6 µmol/plate (0 - 12200 µg/plate) ⁸	Negative ^{2,3}	(Zeiger & Pagano, 1984)	Spot-Test on inhibition of reversion induced by known mutagens. Published non-GLP study of acceptable quality. Limited report of study details and results. Overall, study and results are considered valid.
	Mutation Assay	<i>Saccharomyces sake</i> strain Kyokai no. 7	0.1, 0.15, 0.20% (1000, 1500, 2000 µg/ml)	Negative	(Kojima et al., 1976)	Published study in Japanese (summary and tables with results in English). Validity of the study cannot be evaluated.
	Sister chromatid exchange	Human lymphocytes	0.1 - 10 mM (12.2 to 1220 µg/ml) ⁸	Negative ⁴	(Norppa & Vainio, 1983)	Published non-GLP study of acceptable quality.
2-(4-Hydroxyphenyl)ethan-1-ol [02.166]	Comet assay	PC human prostate cancer cells	0, 10, 50, 100, 250 µM (0, 1.4, 7, 14, 35 µg/ml) ⁹	Negative ⁵	(Quiles et al., 2002)	Published non-GLP study of acceptable quality. Study is considered valid.
(Phenylacetaldehyde [05.030])	Ames reverse mutation assay (preincubation)	<i>S. typhimurium</i> TA98, TA100, TA104 <i>E. coli</i> WP2uvrA/ pKM101	Not specified	Negative ¹	(Kato et al., 1989)	Only abstract reported. Validity of the study cannot be evaluated.
(Phenylacetic acid [08.038])	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, A100, TA1535, TA1537, TA1538	1000 µg/plate ⁷	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No details of study design and results reported. Validity of the study cannot be evaluated.
	Unscheduled DNA synthesis	Rat hepatocytes	500 µg/ml ⁷	Negative	(Heck et al., 1989)	Published non-GLP study. No details of study design and results reported. Validity of the study cannot be evaluated.
	Forward mutation assay	Mouse lymphoma L5178Y TK+/- cells	1000, 1500 µg/ml ⁷	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No details of study design and results reported. Validity of the study cannot be evaluated. It has to be noted, that there was some activity observed in the study even for GRAS substances (for which a negative result was found in the Ames test by the same authors), for which effects of nonphysiological medium conditions on the outcome of the study might be responsible for this. Therefore the validity of the study is questionable.
(Ethyl phenylacetate [09.784])	Ames reverse mutation assay	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537	up to 5000 µg/plate ¹⁰	Negative ¹	(Ishidate et al., 1984)	Published non-GLP study of acceptable quality.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	up to 1000 µg/ml ¹¹	Negative	(Ishidate et al., 1984)	Published non-GLP study of acceptable quality.
	Rec assay	<i>B. subtilis</i> H17 (rec+) and M45 (rec-)	21 µg/disk	Negative	(Oda et al., 1978)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17 (rec+) and M45 (rec-)	20 µg/disk	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Ethyl phenylacetate [09.784]), continued	Mutation assay	<i>E. coli</i> WP2uvrA (trp-)	200 - 1600 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.

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Table IV.4: GENOTOXICITY (<i>in vitro</i>)						
Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
(Isobutyl phenylacetate [09.788])	Ames reverse mutation assay	<i>S. typhimurium</i> TA97, TA102	1, 5, 10, 50 and 100 µg/plate	Negative ¹	(Fujita et al., 1994)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Isoamyl phenylacetate [09.789])	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100	10 µg/plate 50 µg/plate	Negative ¹ Cytotoxic ¹	(Oda et al., 1978)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17 (rec+) and M45 (rec-)	20 µg/disk	Weakly positive ¹²	(Oda et al., 1978)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17 (rec+) and M45 (rec-)	20 µg/disk	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(p-Tolylacetaldehyde [05.042])	Ames reverse mutation assay	<i>S. typhimurium</i> TA100	0.1, 1, 10, 100, 1000 µg/plate	Negative	(Rapson et al., 1980)	Published non-GLP study. Study design and results insufficiently reported. Validity of the study cannot be evaluated.
	SOS Chromtest	<i>E. coli</i> PQ37	Not specified	Negative ⁴	(Ohshima et al., 1989)	Published non-GLP. p-Tolylacetaldehyde has not been analysed per se but after nitrosation (it is unclear to the rapporteur whether the substance has been assayed at all in the study). Due to limited report of experimental details and results the validity of the study cannot be evaluated.
(Isoeugenyl phenylacetate ⁶ [09.710])	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	up to 3600 µg/plate ¹³	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.

¹ With and without S9 metabolic activation.² With S9 metabolic activation.³ Toxic at concentrations from 91.3 µmol/plate.⁴ Without S9 metabolic activation.⁵ At the two highest dose levels evaluated 2-(4-hydroxyphenyl)ethan-1-ol reduced the DNA damage of H₂O₂ treated cells (by 23% at 100 µM and by 40%, at 250 µM)⁶ A phenyl acetate ester structurally related to the candidate chemicals and supporting chemicals, phenethyl alcohol, aldehyde, acid, and related acetals and esters and related substances JECFA (JECFA, 2004a).⁷ Highest inactive dose tested⁸ Calculated based on molecular weight = 122.16⁹ Calculated based on molecular weight = 138.17¹⁰ Six different concentrations used (single concentrations not reported)¹¹ Three different doses used (single doses not reported)¹² D = 2.1 determined (criteria: weakly positive if D ≥ 2, positive if D ≥ 5), using the evaluation criteria of Yoo, 1986, the outcome of the study would have been negative (negative if D < 4, weakly positive if 4 ≤ D < 8, positive if 8 ≤ D < 12, strongly positive if D ≥ 12)¹³ Five different concentrations used (single concentrations not reported)

Phenethyl alcohol, aldehyde, esters, and related phenylacetic acid esters from chemical group 15

In vivo mutagenicity/genotoxicity data are only available for one structurally related substance evaluated by JECFA at the 61st meeting.

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

Table IV.5: GENOTOXICITY (<i>in vivo</i>)							
Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Isoeugenyl phenylacetate ¹ [09.710])	Micronucleus formation assay	Mouse bone marrow cells	i.p.	0, 564, 987 or 1410 mg/kg bw (two applications)	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
	Sex-linked recessive mutation	<i>D. melanogaster</i>	NR	25 mM	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. Study is considered valid.

NR=Not Reported

¹A phenyl acetate ester structurally related to the candidate substances and supporting substances, phenethyl alcohol, aldehyde, acid, and related acetals and esters and related substances JECFA (JECFA, 2004a).

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