European Food Safety Authority

The EFSA Journal (2009) 930, 1-53

Flavouring Group Evaluation 14, Revision 1 (FGE.14Rev1) [1]
Phenethyl alcohol, aldehyde, acetals, carboxylic acid and related esters from chemical group 15 and 22

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC)

(EFSA-Q-2003-157B)

Adopted on 16 May 2007

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel is asked to evaluate 13 flavouring substances in the Flavouring Group Evaluation 14, Revision 1 (FGE.14Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 13 flavouring substances belong to chemical group 15 and 22, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation (FGE) deals with eight phenethyl alcohol derivatives (alcohol, esters and acetals), one phenylacetic acid, one phenethyl aldehyde derivative and three phenylacetals.

Three of the 13 flavouring substances possess a chiral centre and two flavouring substances possess three chiral centres. For two of the substances the stereoisomeric composition has not been specified. The substances have been evaluated irrespective of their chirality. One of the substances can exist as geometrical isomers due to the presence and position of the double bond, but the geometrical isomeric form is given by the name.

Twelve of the 13 flavouring substances are classified into structural class I and one flavouring substance is classified into structural class II according to the decision tree approach presented by Cramer et al. (1978).

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from Commission on Flavouring Group Evaluation 14, Revision 1 (FGE.14Rev1). Phenethyl alcohol, aldehyde, acetals, carboxylic acid and related esters from chemical group 15 and 22.

Ten 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 Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour 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 13 flavouring substances have intakes in Europe from 0.0012 to 33 microgram/*capita*/day, which are well below the threshold of concern for structural class I of 1800 microgram/person/day and for structural class II of 540 microgram/person/day.

All 13 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 13 candidate substances in this FGE. 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 FGE using the Procedure.

It is considered that on the basis of the default MSDI approach these 13 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 5700 microgram/person/day for the 12 flavouring substances from structural class I and for the candidate substance from structural class II the mTAMDI is 3900 microgram/person/day. Thus, the intakes were above the threshold of concern for structural class I of 1800 microgram/person/day and of 560 microgram/person/day for structural class II, except for one candidate substance [FL-no: 05.159] from class I. 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, 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 for 12 of the 13 flavouring substances considered in this Opinion. Therefore, for these 12 substances [FL-no: 02.166, 06.078, 06.080, 08.108, 09.201, 09.620, 09.684, 09.685, 09.686, 09.756, 09.761 and 09.774] 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 13 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 12 of the 13 candidate substances. Identity test and information on chirality is missing for one substance [FL-no: 09.756]. Information on chirality is also missing for [FL-no: 08.108]. Thus, the final evaluation of the materials of commerce cannot be performed for two of the 13 substances [FL-no: 08.108 and 09.756], pending further information. The remaining 11 substances would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

KEYWORDS

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

TABLE OF CONTENT

Summary **1**
Keywords **3**
Background **5**
History of the evaluation **5**
Terms of Reference **5**
Assessment **6**
1. Presentation of the Substances in the Flavouring Group Evaluation 14, Revision 1 6
   1.1. Description 6
   1.2. Stereoisomers 6
   1.3. Natural Occurrence in Food 6
2. Specifications 7
3. Intake data 7
   3.1. Estimated Daily *per Capita* Intake (MSDI Approach) 8
   3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI) 8
4. Absorption, Distribution, Metabolism and Elimination 10
5. Application of the Procedure for the Safety Evaluation of Flavouring Substances 10
6. Comparison of the Intake Estimations based on the MSDI Approach and the mTAMDI Approach 11
7. Considerations of Combined Intakes From Use as Flavouring Substances 12
8. Toxicity 13
   8.1. Acute Toxicity 13
   8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies 13
   8.3. Developmental / Reproductive Toxicity Studies 13
   8.4. Genotoxicity Studies 13
9. Conclusions 15
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 14, Revision 1 ..... **17**
Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) **19**
Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters and Acetals **21**
Table 3: Supporting Substances Summary **23**
Annex I: Procedure for the Safety Evaluation **28**
Annex II: Use Levels / mTAMDI **30**
Annex III: Metabolism **33**
Annex IV: Toxicity **39**
References: **46**
Scientific Panel Members **53**
Acknowledgement **53**

BACKGROUND

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

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

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

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

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

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No of candidate substances
FGE.14	28 April 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1024_en.html	10
FGE.14Rev1	16 May 2007		13

The present revision of FGE.14, FGE.14Rev1, includes the assessment of three additional candidate substances [FL-no: 08.108, 09.756 and 09.774]. New toxicity and metabolism data were provided for [FL-no: 09.774]. Additional information on six substances [FL-no: 06.078, 06.080, 09.201, 09.620, 09.684 and 09.686] was made available since the FGE.14 was published.

TERMS OF REFERENCE

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

ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 14, Revision 1

1.1. Description

The present Flavouring Group Evaluation 14, Revision 1 (FGE.14Rev1), 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 one phenethyl alcohol [FL-no: 02.166], five phenethyl esters [FL-no: 09.201, 09.684, 09.685, 09.686 and 09.774], two phenethyl acetals [FL-no: 06.078 and 06.080], one phenylacetaldehyde [FL-no: 05.159], one phenylacetic acid [FL-no: 08.108] and three phenylacetates [FL-no: 09.620, 09.756 and 09.761]. These 13 flavouring substances belong to chemical group 15 and 22, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000).

The 13 flavouring substances under consideration, with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structures and specifications, are listed in Table 1 and 2a. This group of substances (candidate substances) includes 13 flavouring substances (candidate substances), which are closely related structurally to 37 flavouring substances (supporting substances) evaluated at the 59th JECFA meeting (JECFA, 2002c) in the group of "Phenethyl alcohol, aldehyde, acid and related acetals and esters". The names and structures of the 37 supporting substances are listed in Table 3, together with their evaluation status.

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

1.2. Stereoisomers

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

Three of the 13 candidate substances possess a chiral centre [FL-no: 06.080, 08.108 and 09.686]. Two candidate substances [FL-no: 09.620 and 09.756] possess three chiral centres. For two substances [FL-no: 08.108 and 09.756] the stereoisomeric composition has not been specified (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

Ten out of 13 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 seven of these substances (TNO, 2000).

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.
- Phenethyl benzoate [FL-no: 09.774]: 0.1 mg/kg in bilberry, 0.03 mg/kg in sea buckthorn.

According to TNO three of the substances, *p*-methoxyphenylacetaldehyde [FL-no: 05.159], phenethyl crotonate [FL-no: 09.684] and isobornyl phenylacetate [FL-no: 09.756] have not been reported to occur naturally in any food items (TNO, 2000).

2. Specifications

Purity criteria for the 13 candidate substances have been provided by the Flavour Industry (EFFA, 2003k; EFFA, 2004aa; EFFA, 2004ab; EFFA, 2006e).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the information is adequate for 12 of the 13 candidate substances. Identity test is missing for one substance [FL-no: 09.756]. However, information on chirality is needed for two candidate substances [FL-no: 08.108 and 09.756] (see Section 1.2 and Table 1).

3. Intake data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

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

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

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

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

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

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

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

Average *per capita* intake MSDI is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population[2] (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.14Rev1) the total annual volume of production of the 13 candidate substances for use as flavouring substances in Europe has been reported to be approximately 310 kg (EFFA, 2003l; EFFA, 2004aa; EFFA, 2004ab; EFFA, 2006f) 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 13 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a). Approximately 90 % of the annual volume of production for the candidate substances is accounted for by one flavouring, phenethyl benzoate [FL-no: 09.774]. The estimated daily *per capita* intakes of this candidate substance from use as a flavouring substance is 33 microgram. The daily *per capita* intakes for each of the remaining substances is less than 2 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 13 candidate substances, information on food categories and normal and maximum use levels[3,4,5] were submitted by the Flavour Industry (EFFA, 2003k; EFFA,

[2] *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.*

[3] *"Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).*

2004aa; EFFA, 2004ab; EFFA, 2006e; EFFA, 2007a). The 13 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 1	Dairy products, excluding products of category 2	All 13
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All 13 except [FL-no: 09.774]
Category 3	Edible ices, including sherbet and sorbet	All 13
Category 4.1	Processed fruits	All 13 except [FL-no: 09.774]
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 5	Confectionery	All 13
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 13
Category 7	Bakery wares	All 13
Category 8	Meat and meat products, including poultry and game	All 13
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All 13
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All 13
Category 13	Foodstuffs intended for particular nutritional uses	All 13 except [FL-no: 09.774]
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 13
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 13
Category 15	Ready-to-eat savouries	All 13
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 13 except [FL-no: 09.774]

According to the Flavour Industry the normal use levels for the 13 candidate substances are in the range of 1 - 80 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2003k; EFFA, 2004aa; EFFA, 2004ab; EFFA, 2006e; EFFA, 2007a).

The mTAMDI values for the 12 candidate substances from structural class I (see Section 5) range from 1600 to 5700 microgram/person/day and for the candidate substance from structural class II the mTAMDI is 3900 microgram/person/day.

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

[4] *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).*

[5] *The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).*

4. Absorption, Distribution, Metabolism and Elimination

The eight esters [FL-no: 09.201, 09.620, 09.684, 09.685, 09.686, 09.756, 09.761 and 09.774] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols (see Table 2b), 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 2b and Annex III).

The carboxylic acids, lactic acid, valeric acid, decanoic acid, benzoic acid and crotonic acid resulting from hydrolysis of the candidate esters, 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.

It is anticipated that the candidate carboxylic acid 2-phenylpropionic acid [FL-no: 08.108] is conjugated or partially excreted unchanged.

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 oxidised 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 13 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. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For

comparison of the intake estimations based on the MSDI approach and the mTAMDI approach (see Section 6).

For the safety evaluation of the 13 candidate substances from chemical group 15 and 22 the Procedure as outlined in Annex I was applied. The stepwise evaluations of the 13 substances are summarised in Table 2a.

Step 1

Twelve of the candidate substances of chemical group 15 and 22 [FL-no: 02.166, 05.159, 06.078, 06.080, 08.108, 09.201, 09.620, 09.684, 09.685, 09.686, 09.761 and 09.774] are classified in structural class I and [FL-no: 09.756] is classified in structural class II 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 13 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).

Step A3

The 13 candidate substances have estimated European daily *per capita* intakes (MSDI) from 0.012 to 33 microgram (Table 2a). The intakes are below the threshold of concern of 1800 microgram/person/day for structural class I and of 540 microgram/person/day for structural class II.

Based on results of the safety evaluation sequence these 13 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 12 candidate substances in structural class I based on the mTAMDI range from 1600 to 5700 microgram/person/day. For all of the substances except [FL-no: 05.159] the mTAMDI values are above the threshold of concern for strucural class I of 1800 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

The estimated intake for the candidate substance assigned to structural class II, based on the mTAMDI is 3900 microgram/person/day, which is above the threshold of concern for structural class II of 540 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

Therefore, for 12 substances [FL-no: 02.166, 06.078, 06.080, 08.108, 09.201, 09.620, 09.684, 09.685, 09.686, 09.756, 09.761 and 09.774] of the 13 candidate substances further information is required. This would include more reliable intake data and where required additional toxicological data.

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

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.166	2-(4-Hydroxyphenyl)ethan-1-ol	0.12	2300	Class I	1800
05.159	p-Methoxyphenylacetaldehyde	0.037	1600	Class I	1800
06.078	1,1-Diphenethoxyethane	0.012	3900	Class I	1800
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane	0.012	3900	Class I	1800
08.108	2-Phenylpropionic acid	0.0012	3200	Class I	1800
09.201	Phenethyl valerate	0.012	3900	Class I	1800
09.620	Menthyl phenylacetate	1.5	3900	Class I	1800
09.684	Phenethyl crotonate	0.73	3900	Class I	1800
09.685	2-Phenethyl decanoate	0.037	3900	Class I	1800
09.686	Phenethyl lactate	0.24	3900	Class I	1800
09.761	Pentyl phenylacetate	1.9	3900	Class I	1800
09.774	Phenethyl benzoate	33	5700	Class I	1800
09.756	Isobornyl phenylacetate	0.012	3900	Class II	540

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 FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDIs for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003l; EFFA, 2004aa; EFFA, 2004ab; EFFA, 2006f), the combined estimated daily *per capita* intake as flavourings of the 12 candidate flavouring substances assigned to structural class I from chemical group 15 and 22 is 40 microgram, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day.

The 13 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 2040 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, 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 three candidate substances [FL-no: 06.080, 09.620 and 09.774]. Oral LD_{50} values from three studies in rats were ≥5000 mg/kg body weight (bw) for the three substances.

Twenty-four supporting substances were tested for acute oral toxicity in mouse, rat and guinea pig. The LD_{50} values ranged from 400 mg/kg bw to approximately 15000 mg/kg bw.

The magnitudes of the LD_{50} values indicate that the oral acute toxicity is low for the candidate substances and supporting substances.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

No data on oral, subacute, subchronic and chronic toxicity or carcinogenicity 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].

The repeated dose toxicity studies are summarised in 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.

The developmental / reproductive toxicity studies are summarised in Annex IV, Table IV.3.

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 *in vivo* mutagenicity/genotoxicity data available neither for the candidate substances of the present FGE 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 alcohol[6] (syn. 2-phenylethan-1-ol) [FL-no: 02.019] and ethyl phenylacetate [FL-no: 09.784][7] 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 occur naturally in many foods in milligram range (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 bw/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 *S. 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).

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 13 candidate substances [FL-no: 02.166] in this FGE. 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.

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

[7] *supporting pentyl phenylacetate [FL-no: 09.761], menthyl phenylacetate [FL-no: 09.620].*

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 FGE.

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 13 candidate substances in this FGE.

The genotoxicity data are summarised in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The present Flavouring Group Evaluation (FGE) deals with one phenethyl alcohol, five phenethyl esters, two phenethyl acetals, one phenylacetaldehyde, one phenylacetic acid and three phenylacetates from chemical group 15 and 22.

Three of the 13 candidate substances possess a chiral centre [FL-no: 06.080, 08.108 and 09.686]. Two candidate substances [FL-no: 09.620 and 09.756] possess three chiral centres. For two of the substances [FL-no: 08.108 and 09.756] the stereoisomeric composition has not been specified.

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 the name.

Twelve of the 13 flavouring substances are classified into structural class I and one flavouring substance is classified into structural class II.

Ten of the substances in the present group of 13 substances have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the 13 flavouring substances have intakes in Europe from 0.0012 to 33 microgram/*capita*/day, which are well below the threshold of concern for structural class I of 1800 microgram/person/day and of structural class II of 540 microgram/person/day.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the 12 candidate substances belonging to class I would result in a total intake of approximately 40 microgram/*capita*/day. This value is below the threshold of concern for structural class I of 1800 microgram/person/day. The total combined intake of the candidate and supporting substances in Europe is approximately 2040 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.

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 13 candidate substances in this FGE.

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.

All 13 substances are expected to be metabolised to innocuous substances at the estimated levels of use as flavouring substances.

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

It is considered that on the basis of the default MSDI approach these 13 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 5700 microgram/person/day for the 12 flavouring substances from structural class I and for the candidate substance from structural class II mTAMDI is 3900 microgram/person/day. Thus, the intakes were above the threshold of concern for structural class I of 1800 microgram/person/day and of 560 microgram/person/day for structural class II 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 12 of the 13 flavouring substances [FL-no: 02.166, 06.078, 06.080, 08.108, 09.201, 09.620, 09.684, 09.685, 09.686, 09.756, 09.761 and 09.774] 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 12 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 13 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 12 of the 13 candidate substances. Identity test and information on chirality is missing for one substance [FL-no: 09.756]. Information on chirality is also missing for [FL-no: 08.108]. Thus, the final evaluation of the materials of commerce cannot be performed for two of the 13 substances [FL-no: 08.108 and 09.756], pending further information. The remaining 11 substances would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

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

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 14, Revision 1

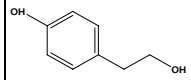
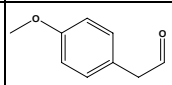
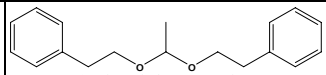
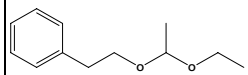
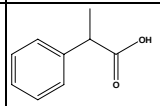
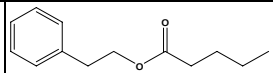
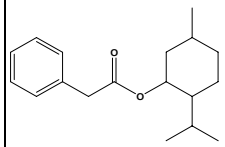
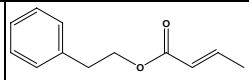
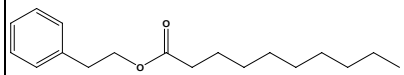
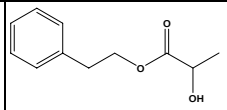
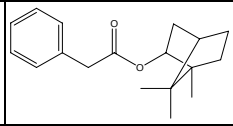
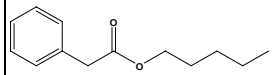
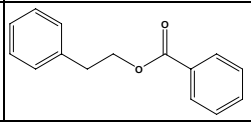
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
02.166	2-(4-Hydroxyphenyl)ethan-1-ol		10226 501-94-0	Solid $C_8H_{10}O_2$ 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 $C_9H_{10}O_2$ 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	Alcohol moiety: 2-phenethyl.
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	Racemate.
08.108	2-Phenylpropionic acid 6)		10164 492-37-5	Liquid $C_9H_{10}O_2$ 150.18	Slightly soluble 1 ml in 1 ml	261 16 MS 95 %	1.517-1.523 1.094-1.100	
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	Alcohol moiety: 2-phenethyl.
09.620	Menthyl phenylacetate		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	Name (±)-menthyl phenylacetate, covering both (+)- and (-)-menthyl ester. Mirror images of the racemic menthyl mixture (trans-1,2-cis-1,5) are covered: (1R,2S,5R)- and (1S,2R,5S). CASrn in Register=(1R,2S,5R)-enantiomer. CASrn to be changed to 1154-92-3.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 14, Revision 1

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
09.684	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	Alcohol moiety: 2-phenethyl, acid moiety: (E)-isomer.
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	
09.686	Phenethyl lactate	 (2-Phenethyl structure shown)	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.	Racemate. Alcohol moiety: 2-phenethyl.
09.756	Isobornyl phenylacetate 6)		566 94022-06-7	Solid $C_{18}H_{24}O_2$ 272.39	Practically insoluble or insoluble 1 ml in 5 ml	432 159 95 %	n.a. n.a.	ID 7).
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	
09.774	Phenethyl benzoate		2860 667 94-47-3	Liquid $C_{15}H_{14}O_2$ 226.28	Very soluble Soluble	331 IR MS 98 %	1.558-1.562 1.092-1.096	

1) Solubility in water, if not otherwise stated.

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

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

7) ID: Missing identification test.

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

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

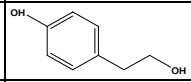
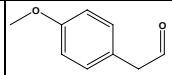
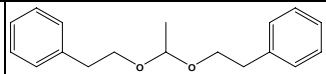
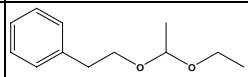
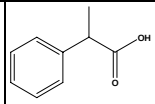
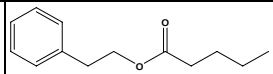
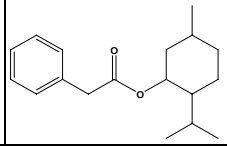
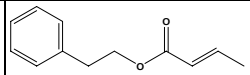
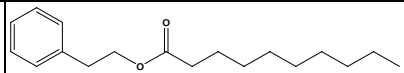
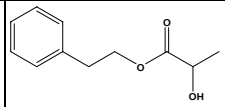
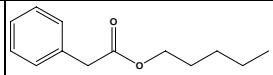
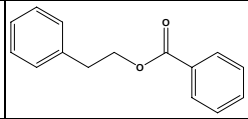
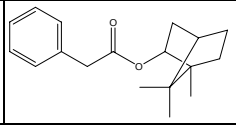
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.166	2-(4-Hydroxyphenyl)ethan-1-ol		0.12	Class I A3: Intake below threshold	4)	6)	
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)	6)	
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane		0.012	Class I A3: Intake below threshold	4)	6)	
08.108	2-Phenylpropionic acid		0.0012	Class I A3: Intake below threshold	4)	7)	
09.201	Phenethyl valerate	 (2-Phenethyl structure shown)	0.012	Class I A3: Intake below threshold	4)	6)	
09.620	Menthyl phenylacetate		1.5	Class I A3: Intake below threshold	4)	6)	
09.684	Phenethyl crotonate	 (2-Phenethyl structure shown)	0.73	Class I A3: Intake below threshold	4)	6)	

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) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.685	2-Phenethyl decanoate		0.037	Class I A3: Intake below threshold	4)	6)	
09.686	Phenethyl lactate	 (2-Phenethyl structure shown)	0.24	Class I A3: Intake below threshold	4)	6)	
09.761	Pentyl phenylacetate		1.9	Class I A3: Intake below threshold	4)	6)	
09.774	Phenethyl benzoate		33	Class I A3: Intake below threshold	4)	6)	
09.756	Isobornyl phenylacetate		0.012	Class II A3: Intake below threshold	4)	7)	

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

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

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

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

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

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

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

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

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

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

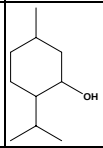
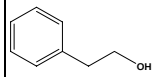
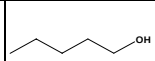
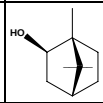
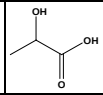
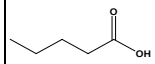
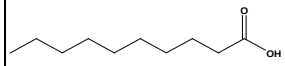
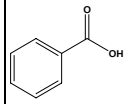
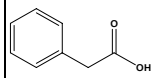
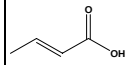
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.015	Menthol 427		No safety concern a) Category A b)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	NOAEL: 380 mg/kg bw/day.
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	
02.059	Isoborneol 1386		No safety concern f) Category B b)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 d) No safety concern g)	No evaluation	At the 46th JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
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 h) 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	

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.011	Decanoic acid 105		Category 1 d) No safety concern e) Category A b)	Class I A3: Intake below threshold	
08.021	Benzoic acid 850		No safety concern c) Deleted b)	Class I A3: Intake below threshold	Substances for which CoE Committee of Experts had no information as to real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992) .
08.038	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)	 (E)-isomer shown		No evaluation	

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

2) No safety concern at estimated levels of intake.

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

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

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

a) (JECFA, 2000a).

b) (CoE, 1992).

c) (JECFA, 2002c).

d) (SCF, 1995).

e) (JECFA, 1999b).

f) (JECFA, 2005c).

g) (JECFA, 1997a).

h) (JECFA, 2002b).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

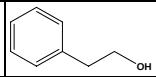
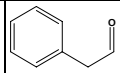
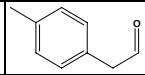
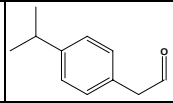
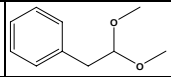
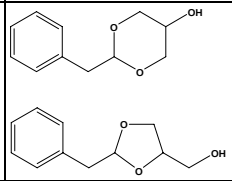
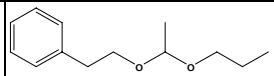
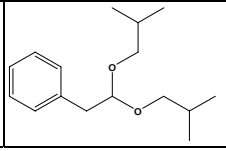
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.019	2-Phenylethan-1-ol		2858 68 60-12-8	987 JECFA specification (JECFA, 2002d)	1200	No safety concern a) Category B b)	
05.030	Phenylacetaldehyde		2874 116 122-78-1	1002 JECFA specification (JECFA, 2002d)	37	No safety concern a) Category B b)	No ADI allocated (JECFA, 1968).
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)	

Table 3: Supporting Substances Summary

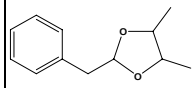
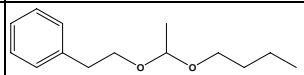
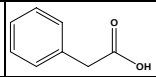
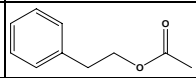
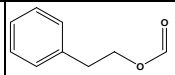
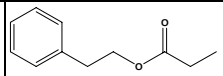
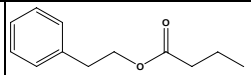
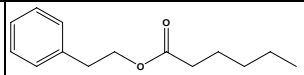
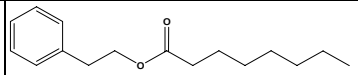
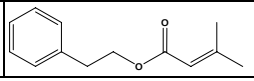
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
06.027	4,5-Dimethyl-2-benzyl-1,3-dioxolan		2875 669 5468-06-4	1005 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to the real use in foodstuffs and/or for which insufficient technological 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)	

Table 3: Supporting Substances Summary

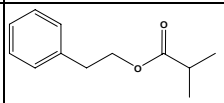
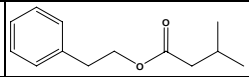
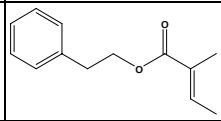
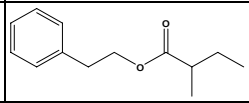
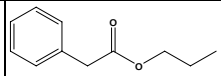
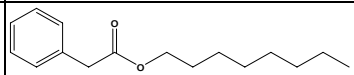
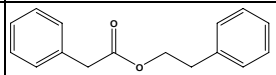
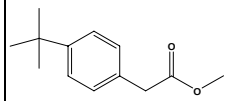
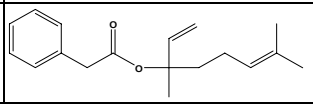
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.427	Phenethyl isobutyrate		2862 302 103-48-0	992 JECFA specification (JECFA, 2002d)	19	No safety concern a) Category 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 the 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)	

Table 3: Supporting Substances Summary

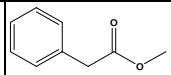
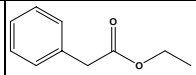
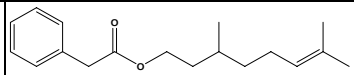
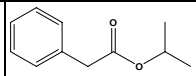
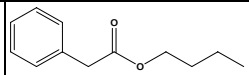
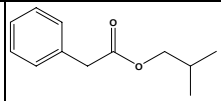
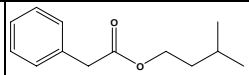
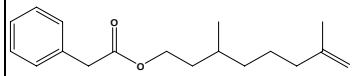
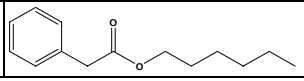
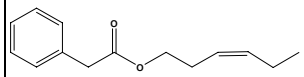
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.783	Methyl phenylacetate		2733 2155 101-41-7	1008 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	No ADI allocated (JECFA, 1968).
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 the 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)	

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
09.805	Hex-3(cis)-enyl phenylacetate		3633 10682 42436-07-7	1016 JECFA specification (JECFA, 2002d)	0.73	No safety concern a)	JECFA evaluated 3-hexenyl phenylacetate (CASrn as in Register). Register CASrn refers to the (Z)-isomer.

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

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

3) No safety concern at estimated levels of intake.

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

a) (JECFA, 2002c).

b) (CoE, 1992).

5) Deleted: Substances for which 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).

ND: No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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

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

- can the flavourings be predicted to be metabolised to innocuous products[8] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[9] (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.

[8] *"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).*

[9] *"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).*

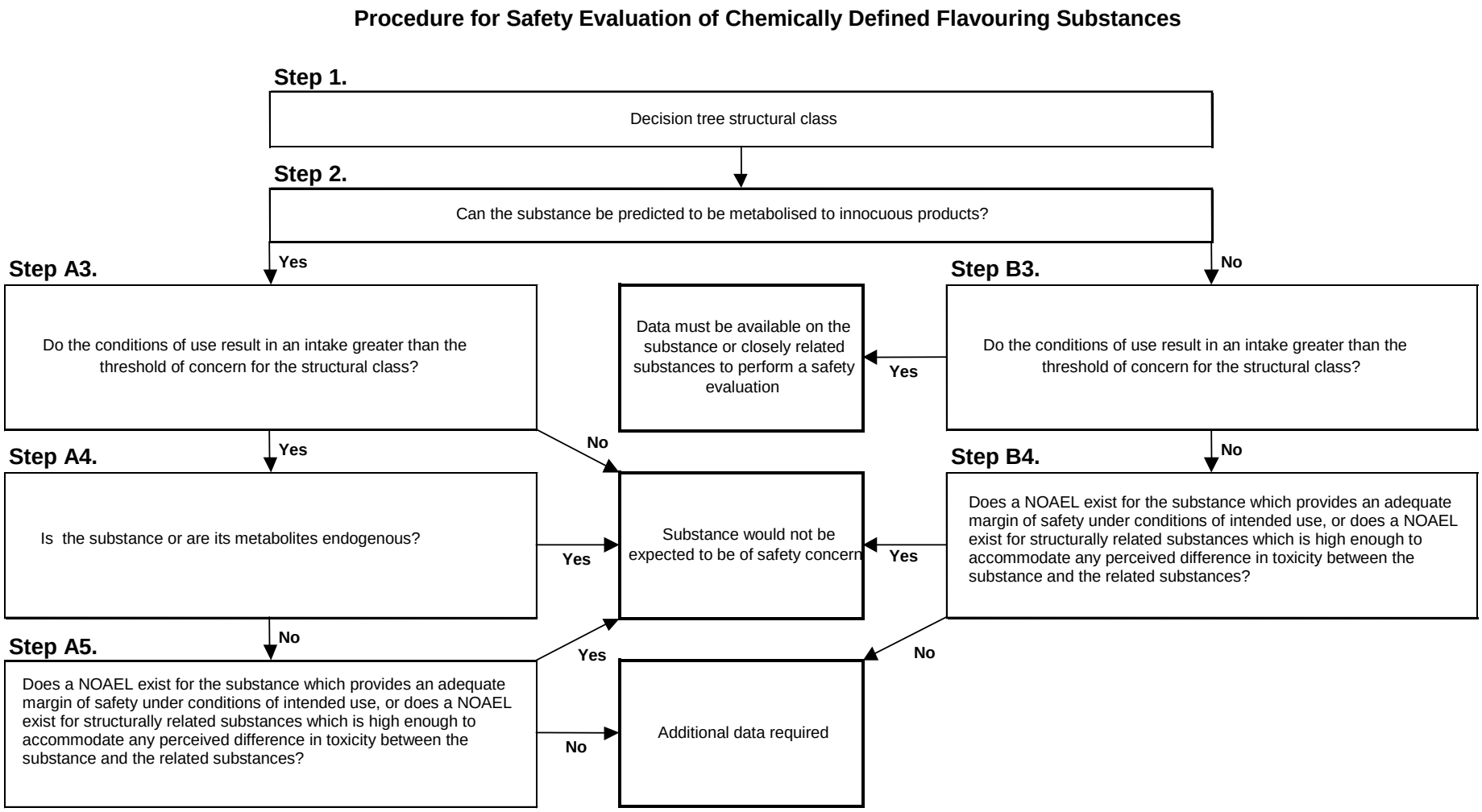


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

The "normal and maximum use levels" are provided by Industry for the 13 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.14Rev1 (EFFA, 2003k; EFFA, 2004aa; EFFA, 2004ab; EFFA, 2006e; EFFA, 2007a).

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 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	- -	10 50	20 100	5 25
05.159	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
06.078	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
06.080	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
08.108	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	10 50	15 75	5 25
09.201	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.620	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.684	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.685	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.686	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.756	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.14Rev1 (EFFA, 2003k; EFFA, 2004aa; EFFA, 2004ab; EFFA, 2006e; EFFA, 2007a).

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
09.761	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.774	2 8	- -	2 80	- -	- -	60 100	1,2 7	2,4 6	1 5	1 10	- -	- -	10 60	- -	6 10	80 100	1 7	- -

II.2 mTAMDI Calculations

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

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

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

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

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

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

	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01	Dairy products, excluding products of category 02.0	Food		
02	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		

04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05	Confectionery			Exception a
06	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07	Bakery wares	Food		
08	Meat and meat products, including poultry and game	Food		
09	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10	Eggs and egg products	Food		
11	Sweeteners, including honey			Exception a
12	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15	Ready-to-eat savouries			Exception b
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values are presented for each of the 13 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003k; EFFA, 2004aa; EFFA, 2004ab; EFFA, 2006e; EFFA, 2007a). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3.)

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.166	2-(4-Hydroxyphenyl)ethan-1-ol	2300	Class I	1800
05.159	p-Methoxyphenylacetaldehyde	1600	Class I	1800
06.078	1,1-Diphenethoxyethane	3900	Class I	1800
06.080	1-Ethoxy-1-(2-phenylethoxy)ethane	3900	Class I	1800
08.108	2-Phenylpropionic acid	3200	Class I	1800
09.201	Phenethyl valerate	3900	Class I	1800
09.620	Menthyl phenylacetate	3900	Class I	1800
09.684	Phenethyl crotonate	3900	Class I	1800
09.685	2-Phenethyl decanoate	3900	Class I	1800
09.686	Phenethyl lactate	3900	Class I	1800
09.761	Pentyl phenylacetate	3900	Class I	1800
09.774	Phenethyl benzoate	5700	Class I	1800
09.756	Isobornyl phenylacetate	3900	Class II	540

ANNEX III: METABOLISM

III.1. Absorption, distribution and excretion

Flavouring Group Evaluation 14, Revision 1 (FGE.14Rev1) includes 13 substances structurally related to phenethyl alcohol, phenethyl aldehyde and phenylacetic acid. Eight of the candidate substances are esters. The chemical group also contains two acetals in which the phenyl groups are localised at the alcohol moieties. An alcohol and aldehyde are also contained in the group of which the first contains a hydroxyphenyl group and the second contains a methoxyphenyl group. An acid is also contained in the 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 eight 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 occure 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*.

One hydrolysis study on the candidate esters is available in which the half-time for *in vitro* hydrolysis of phenetyl benzoate [FL-no: 09.774] in human plasma was 15 minutes (Nielsen & Bundgaard, 1987). 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, menthyl phenylacetate and isobornyl phenylacetate [FL-no: 09.686, 09.201, 09.685, 09.684, 09.761, 09.620 and 09.756] are predicted to undergo hydrolysis to phenethyl alcohol, isoborneol, 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 (Carey, 1992; Streitwieser et al, 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]. The 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-atom on the aldehyde part of the substance. The 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).

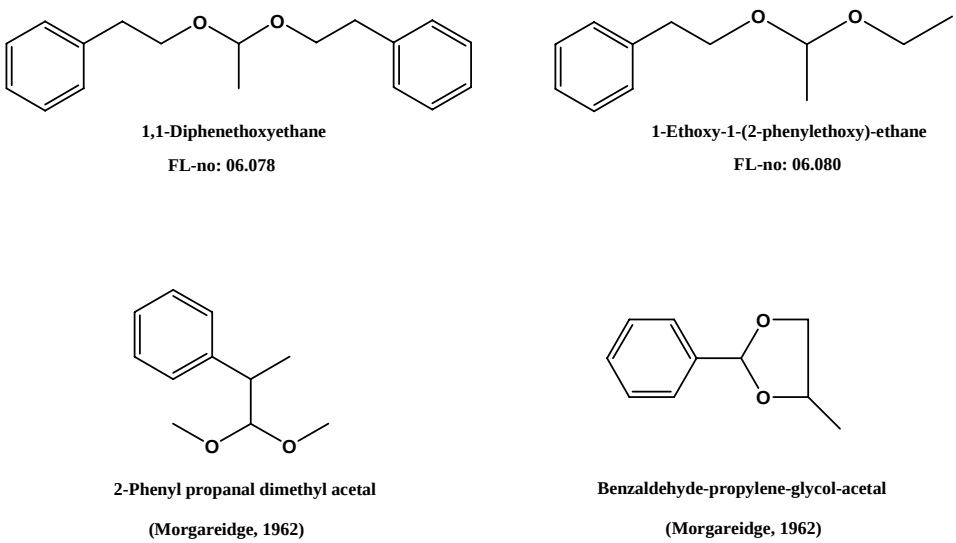


Figure III.1. *Chemical structure of candidate substances and related acetals.*

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 aldehyde 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 (1962a) 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 hydrolysed 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 (Bonanome et al., 2000; Visioli et al., 2000; Miró-Casas et al., 2001a; Miró-Casas et al., 2001b; Tuck et al., 2001; Vissers et al., 2002). 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 (Visioli et al., 2000; Miró-Casas et al., 2001a), 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 the 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 phenetyl alcohol, phenyl acetaldehyde and phenyl acetic acid

The extent to which an alcohol is oxidised 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).

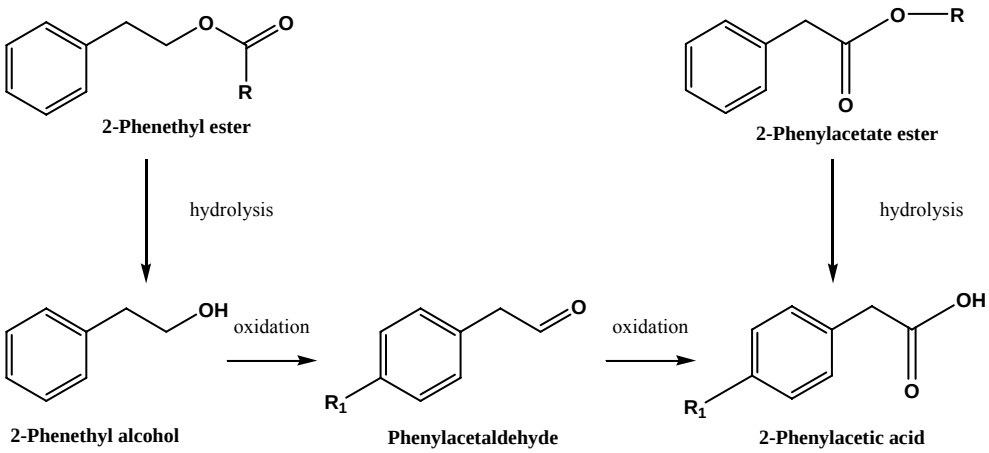


Figure III.2. *Metabolism of 2-phenetyl alcohol, 2-phenylacetaldehyde and 2-phenylacetic acid. R_1 = H- or CH_3-O*

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]. Phenetyl alcohol is first oxidised to phenylacetaldehyde, and thereafter it is further oxidised to phenylacetic acid, with a K_m of 29 nM and 180 microM 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 mmoles/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 eight esters [FL-no: 09.201, 09.620, 09.684, 09.685, 09.686, 09.756, 09.761 and 09.774] included in this FGE 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 hydrolysed almost instantly.

The carboxylic acids resulting from hydrolysis of the candidate chemicals, lactic acid, valeric acid, decanoic acid, crotonic acid and benzoic 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 oxidised to the corresponding carboxylic acid and conjugated to amino acids like glutamine and glycine before excretion in the urine.

It is anticipated that the candidate carboxylic acid, 2-phenylpropionic acid [FL-no: 08.108], is conjugated or partially excreted unchanged.

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 glycuronide 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 candidate substances are expected to be metabolised to innocuous products at the estimated levels of intake.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for three candidate substances of the present FGE from chemical group 15 and 22, 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., 1982a)	
	Rat	M, F	Oral	2509[1]	(Carpenter et al., 1974)	
	Rat	F	Gavage	3100[4]	(Grote & Woods, 1955)	
(Phenethyl formate [09.083])	Rat	M, F	Gavage	3220	(Levenstein, 1972c)	
(Phenethyl acetate [09.031])	Rat	NR	Oral	> 5000	(Moreno, 1973e)	
	Rat	NR	Oral	5200	(Rumyantsev et al., 1987)	
	Rat, Mouse and Guinea pig	M, F	Gavage	3670	(Zaitsev & Rakhmanina, 1974)	
(Phenethyl propionate [09.137])	Rat	M, F	Gavage	3980[5]	(Beroza et al., 1975)	
	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)	
	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)	

Table IV.1: ACUTE TOXICITY

Chemical Name	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(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	(Colaianni, 1967)	
(Citronellyl phenylacetate [09.785])	Rat	NR	Oral	> 5000	(Moreno, 1977o)	
Menthyl phenylacetate [09.620]	Rat	NR	Oral	> 5000	(Moreno, 1982d)	
(p-Tolyacetaldehyde [05.042])	Mouse	NR	Oral	> 5000	(Levenstein, 1975d)	
Phenethyl benzoate [09.774]	Rat	NR	Gavage	5000	(Wohl, 1974i)	

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.*

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for none of the candidate substances of the present FGE from chemical group 15 and 22 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.
(*p*-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.

Developmental and reproductive toxicity data are available for none of the candidate substances of the present FGE from chemical group 15 and 22 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 concentration 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.

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

[2] *Single 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).*

[3] *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).*

In vitro mutagenicity/genotoxicity data are available for one candidate substance of the present FGE from chemical group 15 and 22 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 (*in vitro*)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
(Phenethyl alcohol [02.019])	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (366 µg/plate)[8]	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	*S. typhimurium* 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	*Saccharomyces saké* 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)	*S. typhimurium* TA98, TA100, TA104 *E. coli* WP2*uvr*A/ 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	*S. typhimurium* 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	*S. typhimurium* 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	*B. subtilis* H17 (rec +) and M45 (rec-)	21 µg/disk	Negative	(Oda et al., 1979)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* 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.

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

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
	Mutation assay	*E. coli* WP2*uvr*A (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.
(Isobutyl phenylacetate [09.788])	Ames reverse mutation assay	*S. typhimurium* 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	*S. typhimurium* TA98, TA100	10 µg/plate 50 µg/plate	Negative[1] Cytotoxic[1]	(Oda et al., 1979)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* H17 (rec+) and M45 (rec-)	20 µg/disk	Positive[12]	(Oda et al., 1979)	Study published in Japanese with no English abstract. Data extracted from tables only. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* 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*-Tolyacetaldehyde [05.042])	Ames reverse mutation assay	*S. typhimurium* 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	*E. coli* 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	*S. typhimurium* 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 H_2O_2 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 tested.*

[8] *Calculated based on molecular weight = 122.16.*

[9] *Calculated based on molecular weight = 138.17.*

[10] *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).*

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 (*in vivo*)

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	*D. melanogaster*	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

[1]A 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).

REFERENCES:

Ambrose, A.M., Power, F.W., Sherwin, C.P., 1933. Further studies on the detoxication of phenylacetic acid. J. Biol. Chem. 101, 669-675.

Beroza, M., Inscoe, M.N., Schwartz, P.H., Jr, Keplinger, M.L., Mastri, C.W., 1975. Acute toxicity studies with insect attractants. Toxicol. Appl. Pharmacol. 31, 421-429.

Bonanome, A., Pagnan, A., Caruso, D., Toia, A., Xamin, A., Fedeli, E., Berra, B., Zamburlini, A., Ursini, F., Galli, G., 2000. Evidence of postprandial absorption of olive oil phenols in humans. Nutr. Metab. Cardiovasc. Dis. 10(3), 111-120.

Bottomley, A. M., Ratcliffe, H. E., John, D. M., Anderson, A., Dawe, I. S., 1987. Effect of dietary administration of micro-encapsulated phenylethyl alcohol on pregnancy of the rat (embryotoxicity study). Huntington Research Centre Ltd. SCR 3/851049. 26 October, 1987. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bray, H.G., James, S.P., Thorpe, W.V., 1958. Metabolism of some omega-halogenoalkylbenzenes and related alcohols in the rabbit. Biochem. J. 70, 570-579.

Burdock, G.A., Ford, R.A., Bottomley, A.M., John, D.M., 1987. An evaluation of the teratogenic potential of orally administered phenylethyl alcohol. Toxicologist 7(1), 176.

Carey, F.A., 1992. Aldehydes and ketones. Nucleophilic addition to the carbonyl group. In: Organic Chemistry. McGraw-Hill, Inc., New York, 689-693.

Carpenter, C.P., Weil, C.S., Smyth, H.F., 1974. Range-finding toxicty data. List VIII. Toxicol. Appl. Pharmacol. 28, 313-319.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Colaianni, L.J., 1967. Acute toxicity, eye and skin irritation tests on aromatic compounds. Unpublished data submitted by EFFA to SCF.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

Deslongchamps, P., Dory, Y.L., Li, S., 2000. The relative rate of hydrolysis of a series of acyclic and six membered cyclic acetals, ketals, orthoesters, and orthocarbonates. Tetrahedron 56, 353-357.

EC, 1996. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC, 2000. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) no. 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

EC, 2006. Commission Decision 2006/252/EC of 27 March 2006 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 29.3.2006, L 91, 48.

EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

EFFA, 2003k. Submission 2003-4. Flavouring group evaluation of 12 flavouring substances (candidate chemicals) of the chemical group 15 (Annex I of 1565/2000/EC) structurally related to phenethyl alcohol, aldehyde, acid, and related acetals and esters and related substances [JECFA/WHO FAS 50/59] used as flavouring substances. 9 June 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.22.

EFFA, 2003l. Submission 2003-4. Flavouring group evaluation of 12 flavouring substances (candidate chemicals) of the chemical group 15 (Annex I of 1565/2000/EC) structurally related to phenethyl alcohol, aldehyde, acid, and related acetals and esters and related substances [JECFA/WHO FAS 50/59] used as flavouring substances. 9 June 2003. FLAVIS/8.22. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat.

EFFA, 2004ab. Transfer files for FGE 14 including data for beverages - Submission Dossier 2003-4. Transfer file concerning FL-no: 09.364 and 09.756. Email from Chalot, V. to Gry, J. dated 29 September 2004. Unpublished data submitted from EFFA to FLAVIS Secretariat.

EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

EFFA, 2004aa. Transfer files for FGE 15 including data for beverages - Submission Dossier 2003-6. Transfer file concerning FL-no: 08.108. Email from Chalot, V. to Gry, J. dated 29 September 2004. Unpublished data submitted from EFFA to FLAVIS Secretariat.

EFFA, 2006e. Addendum of one flavouring substance (candidate chemical) to the Flavouring Group Evaluation of the chemical group 15 (Annex I of 1565/2000/EC). Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters used as flavouring substances. August 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.71.

EFFA, 2006f. Addendum of one flavouring substance (candidate chemical) to the Flavouring Group Evaluation of the chemical group 15 (Annex I of 1565/2000/EC). Phenyl ethyl alcohols, phenylacetic acids, related esters, phenoxyacetic acids and related esters used as flavouring substances. August 2006. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.71.

EFFA, 2007a. Email from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.

EFSA, 2004a. Minutes of the 7th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.eu.int/science/afc/afc_meetings/502_en.html

Engel, K-H., 2003. Personal communication to the FLAVIS working group. 14 November, 2003.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://europa.eu.int/comm/eurostat/newcronos/queen/display.do?screen=detail&language=en&product=YES&root=YES/yearlies/c/ca/caa/caa10000. August 13, 2004.

Fassett, D.W., 1963. Aldehydes and acetals. In Industrial Hygiene And Toxicology, 2nd Ed. vol. II, pp. 1968-1969.

Florin, I., Rutberg, L., Curvall, M., Enzell, C.R., 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology. 18, 219-232.

Fujita, H., Aoki, N., Sasaki, M., 1994. [Mutagenicity test of food additives with Salmonella typhimurium TA97 and TA102 (IX*)]. Ann. Rep. Tokyo Metrop. Res. Lab. Public Health 45, 191-199. (In Japanese)

Gregus, Z., Fekete, T., Varga, F., Klaassen, C.D., 1993. Dependence of glycine conjugation on availability of glycine: role of the glycine cleavage system. Xenobiotica 23, 141-153.

Grote, I.W., Woods, M., 1955. Beta-phenethyl alcohol as a preservatives for ophthalmic solutions. J. Am. Pharm. Assoc., Sci. Ed. 44, 9-11.

Grundschober, F., 1977. Toxicological assessment of flavouring esters. Toxicology 8, 387-390.

Hagan, E.C., Hansen, W.H., Fitzhugh, O.G., Jenner, P.M., Jones, W.I., Taylor, J.M., Long, E.L., Nelson, A.A., Brouwer, J.B., 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet. Toxicol. 5(2), 141-157.

Heck, J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B., Curren, R.D., 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1), 257-272.

Heymann, E., 1980. Carboxylesterases and amidases. In: Jakoby, W.B. (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

Ishidate, M.Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22(8), 623-636.

James, M.O., Smith, R.L., 1973. The conjugation of phenylacetic acid in phenylketonurics. Eur. J. Clin. Pharmacol. 5(4), 243-246.

James, M.O., Smith, R.L., Williams, R.T., Reidenberg, M., 1972. The conjugation of phenylacetic acid in man, sub-human primates and some non-primate species. Proc. R. Soc. Lond. B. 182(1066), 25-35.

JECFA, 1968. 11. Report: 11th Report of the Joint FAO/WHO Expert Committee on Food Additives. Report: WHO Technical Report Series, no. 383.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA, 2000a. Evaluation of certain food additives. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.

JECFA, 2002b. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 909. Geneva, 5-14 June 2001.

JECFA, 2002c. Evaluation of certain food additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 913. Geneva, 4-13 June 2002.

JECFA, 2002d. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Commitee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Add. 10.

JECFA, 2003a. Safety evaluation of certain food additives. Fifty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.

JECFA, 2004a. Evaluation of certain food additives. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 922. Rome, 10-19 June 2003.

JECFA, 2005c. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.

Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., Fitzhugh, O.G., 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2, 327-343.

Johannsen, E., Purchase, I.F.H., 1969. Kaffircorn malting and brewing studies. XXI The effect of fuel oils of bantu beer on rat liver. S. A. Med. J. 43(12), 326-328.

Kato, F., Araki, A., Nozaki, K., Matsushima, T., 1989. Mutagenicity of aldehydes and diketones. Mutat. Res. 216, 366-367.

Keating, J.W., 1972c. Acute toxicity study in rats and rabbits with phenylacetic acid. Biological Science Laboratories. June 6, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Klyosov, A. A., 1996. Kinetics and specificity of human liver aldehyde dehydrogenases toward aliphatic, aromatic and fused polycyclic aldehydes. Biochemistry 35(14), 4457-4467.

Kojima, M., Katohgi, Y., Hatae, K., 1976. Induction of a respiration-deficient mutant of Saccharomyces saké by phenyl propanols and related compounds. J. Ferment. Technol. 54(1), 11-15. (In Japanese)

Koss, F.W., Lamprecht, W., 1968. Experimental investigations in choleresis. Eur. J. Pharmacol. 4(2), 215-223.

Kreevoy, M.M., Taft Jr., R.W., 1955a. The evaluation of inductive and resonance effects on reactivity. I. Hydrolysis rates of acetals of non-conjugated aldehydes and ketones. J. Am. Chem. Soc. 77, 5590-5595.

Levenstein, I., 1972c. Acute toxicity studies in rats and rabitts. Phenethyl formate, assay no. 30973, February 16, 1973. Iso cyclo citral, assay no. 30698, January 9, 1973. Iso cyclo citral, assay no. 30698, February 16, 1973. Hydroxycitronellol, assay no. 30966, January 9, 1973. Hydroxycitronellol, assay no. 30966, February 16, 1973. Isobutyl benzoate, assay no. 30697, January 10, 1973. Isobutyl benzoate, assay no. 30697, February 16, 1973. Methyl acetate racemic, assay no. 30970, January 10, 1973. Methyl acetate racemic, assay no. 30970, February 16, 1973. Menthone racemic pure, assay no. 30969, January 10, 1973. Menthone racemic pure, assay no. 30969, February 16, 1973. Methylphenylcarbinyl propionate, assay no. 30971, January 17, 1973. Methylphenylcarbinyl propionate, assay no. 30971, February 16, 1973. Rhodinyl acetate, assay no. 30975, January 19, 1973. Leberco Laboratories, Inc. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levenstein, I., 1974d. Acute oral toxicity (rat - 5 gms./kg. body weight dose). Dermal toxicity (rabbit - 5 gms./kg. body weight dose). Phenyl ethyl n-butyrate. Leberco Laboratories, Inc. Assay no. 44579. May 30, 1975. Unpublished data submitted by EFFA to SCF.

Levenstein, I., 1974e. Acute oral toxicity (rat - 5 gms/kg. body weight dose). Dermal toxicity (rabbit - 5 gms./kg. body weight dose). Phenyl ethyl tiglate. Leberco Laboratories, Inc. Assay no. 44582. July 18, 1974. Unpublished data submitted by EFFA to SCF.

Levenstein, I., 1975d. Acute oral toxicity (mice - 5 gms./kg. body weight dose). Dermal toxicity (rabbits - 5 gms./kg. body weight dose). p-Tolyacetaldehyde. Leberco Laboratories, Inc. Assay no. 53808. May 30, 1975. Unpublished data submitted by EFFA to SCF.

Longland, R.C., Shilling, W.H., Gangolli, S.D., 1977. The hydrolysis of flavouring esters by artificial gastrointestinal juices and rat tissue preparations. Toxicology 8, 197-204.

Maganova, N.B., Zaitsev, A.N., 1973. A study of the embryotoxic action of some synthetic food flavorings (Phenylacetic acid, phenylethanol, and cinnamic alcohol). Voprosy Pitani JA 4, 50-54.

Mallory, V.T., Naismith, R.W., Matthews, R.J., 1982a. Acute oral toxicity study in rats (14 days) PH 402-IFF-018-82. Phenethyl alcohol. Pharmakon Research International, Inc. December 27, 1982. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Mankes, R.F., LeFevre, R., Bates, H., Abraham, R., 1983. Effects of various exposure levels of 2-phenylethanol on fetal development and survival in Long-Evans rats. J. Toxicol. Environ. Health 12, 235-244.

Mankes, R. F., LeFevre, R., Abraham, R., 1984. Reproductive effects of some solvent alcohols: evaluations of structure-activity relationships in 2-substituted ethanols. J. Am. Coll. Toxicol. 3(2), 166.

Mankes, R. F., LeFevre, R., Renak, V., Fiesher, J., Abraham, R., 1985. Reproductive effects of some solvent alcohols with differing partition coefficients. Teratology 31(3), 67A.

Martini, R., Murray, M., 1996. Rat hepatic microsomal aldehyde dehydrogenase. Identification of 3- and 4-substituted aromatic aldehydes as substrates of the enzyme. Chem. Res. Toxicol. 9, 268-276.

Miró-Casas, E., Farre Albaladejo, M., Covas, M.I., Rodriguez, J.O., Menoyo Colomer, E., Lamuela Raventós, R.M., de la Torre, R., 2001a. Capillary gas chromatography-mass spectrometry quantitative determination of hydroxytyrosol and tyrosol in human urine after olive oil intake. Anal. Biochem. 294(1), 63-72.

Miró-Casas, E., Farré Albadalejo, M., Covas, M.I., Fitó Colomer, M., Lamuela Raventós, R.M., de la Torre Fornell, R., 2001b. Tyrosol bioavailability in humans after ingestion of virgin olive oil. Clin Chem. 47(2), 341-343.

Moldave, K., Meister, A., 1957. Enzymic acylation of glutamine by phenylacetic acid. Biochim. Biophys. Acta 24, 654-655.

Moreno, O.M., 1972c. Acute oral toxicity in rats. Compound 72-3-6 (phenyl acetaldehyde glyceryl acetal). Toxicological Resources. Project no. 698-72. March 14, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1972d. Single dose toxicity in rats. Compound no. 72-3-6 (phenyl acetaldehyde glyceryl acetate). Toxicological Resources. Project no. 506-72. February 22, 1972. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973e. Acute oral toxicity in rats. Phenyl ethyl acetate. MB Research Laboratories, Inc. May 14, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973f. Acute oral toxicity in rats. Dermal toxicity in rabbits. Phenethyl propionate. MB Research Laboratories, Inc. Project no. MB 72-26. Date 2/1/73. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973g. Acute oral toxicity in rats. Dermal toxicity in rabbits. Ethyl phenyl acetate. MB Research Laboratories, Inc. Project no. MB 72-16. Date 2/1/73. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973h. Acute oral toxicity in rats. Dermal toxicity in rabbits. Isobutyl phenylacetate. MB Research Laboratories, Inc. Project no. MB 73-97. May 18, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1974f. Acute oral toxicity in rats. Dermal toxicity in rabbits. Hyacinth body (1-ethoxy-1(2-phenylethoxy)ethane). MB Research Laboratories, Inc. Project no. MB 74-673. December 11, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1974g. Acute oral toxicity in rats. Dermal toxicity in rabbits. Methyl phenyl acetate. MB Research Laboratories, Inc. Project no. MB 74-572. June 28, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1974h. Acute oral toxicity in rats. Dermal toxicity in rabbits. Linalyl phenyl acetate. MB Research Laboratories, Inc. Project no. MB 74-594. August 22, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1976m. Acute oral toxicity in rats. Dermal toxicity in rabbits. Isoamyl phenylacetate. MB Research Laboratories, Inc. Project no. MB 76-1230. August 2, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1976n. Acute oral toxicity in rats. Dermal toxicity in rabbits. cis-3-Hexenyl phenylacetate. MB Research Laboratories, Inc. Project no. MB 76-1275. September 7, 1976. Unpublished data submitted by EFFA to SCF.

Moreno, O.M., 1977m. Acute oral toxicity in rats. Dermal toxicity in rabbits. Phenyl acetaldehyde pure. MB Research Laboratories, Inc. Project no. MB 76-1460. February 7, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977n. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Phenylacetate rhodinyle (rhodinyl phenylacetate). MB Research Laboratories, Inc. Project no. MB 77-1934. October 7, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977o. Acute oral toxicity in rats. Dermal toxicity in rabbits. Citronellyl phenylacetate. MB Research Laboratories, Inc. Project no. MB 77-1683. Date 5/20/77. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1979c. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Acetaldehyde phenylethyl-n-propyl acetal. MB Research Laboratories, Inc. Project no. MB 77-3417. Date 3/22/79. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1980i. Oral toxicity in rats. Dermal toxicity in rabbits. Butyl phenyl acetate. MB Research Laboratories, Inc. Project no. MB 80-4559. Date 6/25/80. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1982d. Oral toxicity in rats. Dermal toxicity in rabbits. 1-Menthyl phenylacetate. MB Research Laboratories, Inc. Project no. MB 82-6241. Date 9/29/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1982e. Oral toxicity in rats. Dermal toxicity in rabbits. Phenethyl alcohol (musk ambrette 5% in PEA). MB Research Laboratories, Inc. Project no. MB 82-5976. Date 6/08/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1982f. Oral toxicity in rats. Dermal toxicity in rabbits. Phenethyl alcohol (musk ambrette 20% in PEA). MB Research Laboratories, Inc. Project no. MB 82-5977. Date 6/08/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1982g. Oral toxicity in rats. Dermal toxicity in rabbits. Phenylethyl 2-methylbutyrate. MB Research Laboratories, Inc. Project no. MB 82-6244. Date 9/29/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Morgareidge, K., 1962a. In vitro digestion of four acetals. Food and Drug Research Laboratories, Inc. Lab. no. 83179. August 7, 1962. Unpublished report submitted by EFFA to SCF.

Nielsen, N.M., Bundgaard, H., 1987. Prodrugs as drugs delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: A reference system for designing prodrug esters of carboxylic acid agents. Int. J. Pharm. 39, 75-85.

Norppa, H., Vainio, H., 1983. Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. Mutat. Res. 116, 379-387.

Oda, Y., Hamono, Y., Inoue, K., Yamamoto, H., Niihara, T., Kunita, N., 1979. [Mutagenicity of food flavors in bacteria]. Shokuhin. Eisei. Hen. 9, 177-181. (In Japanese)

Ohshima, H., Friesen, M., Malaveille, C., Brouet, I., Hautefeuille, A., Bartsch, H., 1989. Formation of direct-acting genotoxic substances in nitrosated smoked fish and meat products: Identification of simple phenolic precursors and phenyldiazonium ions as reactive products. Food Chem. Toxicol. 27(3), 193-203.

Palmer, A.K., Bottomley, A. M., Ratcliffe, H.E. Clark, R., John, D.M., 1986. Effect of phenylethyl alcohol (PEA) on pregnancy of the rat. Huntingdon Research Center. RIF 5/85705. 3. April, 1986. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Pchelintsev, V.V., Sokolov, A.Y., Zaikov, G.E., 1988. Kinetic principles and mechanisms of hydrolytic degradation of mono- and polyacetals - a review. Polymer Degradation Stabil. 21, 285-310.

Pietruszko, R., Crawford, K., Lester, D. 1973. Comparison of substrate specificity of alcohol dehydrogenases from human liver, horse liver and yeast towards saturated and z-enoil alcohols and aldehydes. Arch. Biochem. Biophys. 159, 50-60.

Posternak, N.M., Linder, A., Vodoz, C.A., 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 7, 405-407.

Quiles, J.L., Farquharson, A.J., Simpson, D.K., Grant, I., Wahle, K.W., 2002. Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells. Br. J. Nutr. 88(3), 225-234.

Rapson, W.H., Nazar, M.A., Butzky, V.V., 1980. Mutagenicity produced by aqueous chlorination of organic compounds. Bull. Environ. Contam. Toxicol. 24, 590-596.

Ridgway, P., 1986. Rat teratology study. ORP 178. Toxicol Laboratories Limited. Ref. no. TAL/9/86. June 1986. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Rumyantsev, G. I., Novikov, S. M., Fursova, T. N., Kochetkova, T. A., Ivanov, IuV., 1987. [Experimental study of the toxic properties of phenylethyl alcohol and phenylethyl acetate]. Gig. Sanit 10, 83-84. (In Russian)

SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev.2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF, 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Seakins, J.W.T., 1971. The determination of urinary phenylacetylglutamine as phenylacetic acid. Studies on its origin in normal subjects and children with cystic fibrosis. Clin. Chim. Acta 35, 121-131.

Shelanski, M.V., Moldovan, M., 1971b. Acute oral toxicity in rats. Phenyl ethyl isobutyrate. Food and Drug Research Laboratories, Inc. I.B.L. no. 10358-F. 26 November, 1971. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Shelanski, M.V., Moldovan, M., 1971c. Acute oral toxicity study in rats. Phenylacetaldehyde dimethyl acetal. Food and Drug Research Laboratories, Inc. I.B.L. no. 10361-F. November 14, 1971. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Streitwieser, A., Heathcock, C.H., Kosower, E.M., 1992. Aldehydes and Ketones. In: Corey, P.F. (Ed.). Introduction to organic chemistry. 4th Ed. Macmillan Publishing Company, New York, pp. 394-453.

Teuchy, H., Quatacker, G., Wolf, G., Van Sumere, C.F., 1971. Quantitative investigation of the hippuric acid formation in the rat after administration of some possible aromatic and hydroaromatic precursors. Arch. Intern. Physiol. Biochem. 79, 573-587.

Thierfelder, H., Schempp, E., 1917. Behavior of benzpylpropionic acid, phenylethyl alcohol and phenoxyacetic acid in the body of men and dogs. Arch. Ges. Physiol. 167, 280-288.

TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Tuck, K.L., Freeman, M.P., Hayball, P.J., Stretch, G.L., Stupans, I., 2001. The *in vivo* fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. J. Nutr. 131(7), 1993-1996.

Visioli, F., Galli, C., Bornet, F., Mattei, A., Patelli, R., Galli, G., Caruso, D., 2000. Olive oil phenolics are dose-dependently absorbed in humans. FEBS Lett. 468(2-3), 159-160.

Vissers, M.N., Zock, P.L., Roodenburg, A.J., Leenen, R., Katan, M.B., 2002. Olive oil phenols are absorbed in humans. J Nutr. 132(3), 409-417.

Wild, D., King, M.T., Gocke, E., Eckhard, K., 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food Chem. Toxicol. 21(6), 707-719.

Wohl, A.J., 1974i. Acute oral toxicity (Rat - 5 gms/kg body weight dose). Dermal toxicity (Rabbit - 5 gms/kg body weight dose). Phenyl Ethyl Benzoate. Biological Science Laboratories. April 2, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Yamaguchi, T., Caldwell, J., Farmer, P.B., 1994. Metabolic fate of [3H]-l-menthol in the rat. Drug Metab. Disposition 22(4), 616-624.

Yoo, Y.S., 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka City Med. J. 34(3-4), 267-288. (In Japanese)

Zaitsev, A.N., Maganova, N.B., 1975. [Embryotoxic effects of some aromatizers for food products]. Vopr. Pitan. 3, 64-68.

Zaitsev, A.N., Rakhmanina, N.L., 1974. Some data on the toxic properties of phenylethyl and cinnamyl alcohol derivatives. Vopr. Pitan. 5, 48-53. (In Russian)

Zeiger, E., Pagano, D.A., 1984. Suppressive effects of chemicals in mixture on the Salmonella plate test response in the absence of apparent toxicity. Environ. Mutag. 6, 683-694.

Zeller, Hoffmann, 1974. [Acute toxicity studies on phenethylalcohol]. Medizinisch-Biologische Forschungslaboratorien. Date 18.9.73. Unpublished data submitted by EFFA to FLAVIS Secretariat. (In German)

SCIENTIFIC PANEL MEMBERS

Fernando Aguilar, Herman Nybro Autrup, Susan Barlow, Laurence Castle, Riccardo Crebelli, Wolfgang Dekant, Karl-Heinz Engel, Nathalie Gontard, David Michael Gott, Sandro Grilli, Rainer Gürtler, John Christian Larsen, Jean-Charles Leblanc, Catherine Leclercq, F. Xavier Malcata, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Ivonne Magdalena Catharina Maria Rietjens, Paul P. Tobback, Fidel Toldrá.

ACKNOWLEDGEMENT

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food wishes to thank Trine Husøy, Judith Amberg-Müller, Karl-Heinz Engel, Harriet Wallin, Jørn Gry, Vibe Beltoft, Karin Nørby, Krestine Greve and Trine Klein Reffstrup for their contribution to the draft opinion.