SCIENTIFIC OPINION

Flavouring Group Evaluation 16, Revision 2 (FGE.16Rev2):

Aromatic ketones from chemical group 21[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to 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 was requested to evaluate seven flavouring substances in the Flavouring Group Evaluation 16, Revision 2 (FGE.16Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These seven flavouring substances belong to chemical group 21, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation (FGE) deals with seven aromatic ketones.

One of the seven candidate substances can exist as an optical isomer.

Six of the seven flavouring substances are classified into structural class I and one is classified into structural class III.

Five out of the seven flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily 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

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2 Panel members Arturo Anadon, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle

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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 seven flavouring substances in this group have intakes in Europe from 0.0012 to 0.85 microgram/*capita*/day, which are below the threshold of concern values for structural class I (1800 microgram/person/day) and structural class III (90 microgram/person/day).

Overall, the limited data available do not allow a final assessment of genotoxicity. From the data available there is some indication of genotoxic potential for two of the supporting substances (1-phenylethan-1-ol [FL-no: 02.064] and acetophenone [FL-no: 07.004]). However, taking into consideration metabolism and carcinogenicity data on the candidate and supporting substances, the positive *in vitro* results do not preclude the evaluation of the candidate substances through the Procedure.

It can be anticipated that six candidate substances 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], 3-Hydroxy-4-phenylbutane-2-one [FL-no: 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are metabolised to innocuous products at the estimated levels of intake. This cannot be anticipated for alpha-methyl naphthyl ketone [FL-no: 07.214].

Based on a No Observed Adverse Effect Level (NOAEL) of 33 mg/kg body weight (bw) for the structurally related substance methyl 2-naphthyl ketone [FL-no: 07.013] a margin of safety of approximately $2x10^9$ could be estimated for the flavouring substance alpha-methyl naphthyl ketone [FL-no: 07.214]. Thus, alpha-methyl naphthyl ketone is not expected to be of safety concern at its estimated level of intake as flavouring substance.

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

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

When the estimated intakes were based on the mTAMDI approach they were 1600 microgram/person/day for five of the six flavouring substances belonging to structural class I and 2100 microgram/person/day for [FL-no: 07.254]. Thus, the intakes were below the threshold of concern for structural class I of 1800 microgram/person/day for five of the six candidate substances belonging to structural class I. For the alpha-methyl naphthyl ketone [FL-no: 07.214] belonging to structural class III the intake was above the threshold of concern of 90 microgram/person/day for structural class III. The five substances which have mTAMDI intake estimates below the threshold of concern for structural class I, are also expected to be metabolised to innocuous products.

Thus, for two of the seven flavouring substances considered in this Opinion, the intake, estimated on the basis of the mTAMDI, exceeds the relevant threshold for its structural class, to which the flavouring substance has been assigned. Therefore, for alpha-methyl naphthyl ketone [FL-no: 07.214] and for 2-methoxy-acetophenone [FL-no: 07.254] more reliable exposure data are required. On the

basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the seven candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity have been provided for six of the seven materials of commerce. For one candidate substance [FL-no: 07.242], the optical isomerism has not been specified. Thus, the final evaluation of the material of commerce cannot be performed for this substance, pending further information on chirality.

The remaining six substances [FL-no: 07.193, 07.194, 07.195, 07.214, 07.254 and 07.259] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

KEYWORDS

Aryl ketones; aromatic ketones; alkanons; flavourings; safety.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/163/EC (EC, 2009a). 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, 2000a), 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 Community 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.16	2 March 2006	http://www.efsa.europa.eu/en/science/afc/afc_opinions.html	4
FGE.16Rev1	16 May 2007		6
FGE.16Rev2	26 March 2009		7

The present Revision of FGE.16, FGE.16Rev2, includes the assessment of one additional candidate substance [FL-no: 07.252]. No toxicity and/or metabolism data were provided for this substance.

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 Community list according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). 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 Flavouring Group Evaluation 16 Revision 2

1.1. Description

The present Flavouring Group Evaluation 16, Revision 2 (FGE.16Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure - shown in schematic form in Annex I), deals with seven aromatic ketones from chemical group 21, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The seven flavouring substances under consideration, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

The present FGE consists of seven aromatic ketones 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], alpha-methyl naphthyl ketone [FL-no: 07.214], 3-hydroxy-4-phenylbutane-2-one [FL-no: 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] which all contain the ketone group in an aliphatic side chain. One of the ketones contains a naphthyl group instead of a phenyl group.

The seven flavouring substances (candidate substances) are closely related structurally to 30 flavouring substances (supporting substances) evaluated at the 57th meeting of the Joint FAO/WHO Expert Committee on Food Additives (the JEFCA) in the group “Aromatic substituted secondary alcohols, ketones and related esters” (JECFA, 2002b). These substances, with the respective structural formulas, FEMA, CoE and CAS register numbers, evaluation status by Scientific Committee on Food (SCF), the JECFA and CoE and the European Maximised Survey-derived Daily Intake (MSDI) values are listed in Table 3.

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

One of the seven candidate substances can exist as an optical isomer [FL-no: 07.242]

1.3. Natural Occurrence in Food

Five out of the seven candidate substances have been reported to occur in one or more of the following food items: avocado, beef, cheese, cocoa, boiled egg, brandy, rum, wine, malt, peanut, pork liver, tomato, wild rice, juice (passion fruit), papaya, peas, pepper, and raspberry (TNO, 2000). Quantitative data on the natural occurrence in food have been reported for three of these substances. These reports are:

- 1-Phenylpropan-2-one [FL-no: 07.195]: up to 0.01 mg/kg in passion fruit juice.
- 3-Hydroxy-4-phenylbutane-2-one [FL-no: 07.242]: up to 0.04 mg/kg in red and white wine.

- 2-Methyl-acetophenone [FL-no: 07.259]: 0.003 mg/kg in papaya.

According to TNO one of the substances, alpha-methyl naphthyl ketone [FL-no: 07.214] has not been reported to occur naturally in any food items (TNO, 2000).

2. Specifications

Purity criteria for the seven substances have been provided by the Flavour Industry (EFFA, 2003o; Flavour Industry, 2006d; Flavour Industry, 2007d) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for six of the seven candidate substances. For one substance [FL-no: 07.242] information on chirality is needed (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 by 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, 2000a). 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 intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These 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 population[4] (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).

The total annual volume of production of the seven candidate substances in the present Flavouring Group Evaluation (FGE.16Rev2) from use as flavouring substances in Europe has been reported to be approximately 12 kg (EFFA, 2003p; Flavour Industry, 2005e; Flavour Industry, 2006d). Nearly all of this amount is accounted for by three of these flavouring substances: 1-phenylbutan-1-one [FL-no: 07.193] 2 kg per year, 1- phenylpropan-2-one [FL-no: 07.195] 7 kg per year and 2-methoxy-acetophenone [FL-no: 07.254] 3 kg per year. For 26 of the 30 supporting substances the total annual volume of production is 3700 kg in Europe (JECFA, 2002b). The annual volumes of production in Europe for four of the supporting substances [FL-no: 07.070, 09.189, 09.200 and 09.501] were not reported.

On the basis of the annual volumes of production reported for the seven candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated. The estimated daily *per capita* intake of 1-phenylbutan-1-one from use as a flavouring substance is 0.24 microgram, that of 1-phenylpropan-2-one is 0.85 microgram and that of and 2-methoxy-acetophenone is 0.37 microgram. For the remaining four substances 4-phenylbutan-2-one, alpha-methyl naphthyl ketone, 3-hydroxy-4-phenylbutane-2-one and 2-methyl-acetophenone the estimated daily *per capita* intake is less than 0.016 microgram for each (Table 2).

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 seven candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2003o; Flavour Industry, 2005e; Flavour Industry, 2006d; EFFA, 2007a; Flavour Industry, 2007d). The seven 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, 2000a), 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.

[4] 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.

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

[6] 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).

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

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All seven
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All seven
03.0	Edible ices, including sherbet and sorbet	All seven
04.1	Processed fruits	All seven except [FL-no: 07.254]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 07.259]
05.0	Confectionery	All seven
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All seven except [FL-no: 07.254]
07..0	Bakery wares	All seven
08.0	Meat and meat products, including poultry and game	All seven except [FL-no: 07.254]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All seven except [FL-no: 07.254]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All seven
13.0	Foodstuffs intended for particular nutritional uses	All seven except [FL-no: 07.254]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All seven
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All seven
15.0	Ready-to-eat savouries	All seven
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All seven except [FL-no: 07.254]

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 1-10 mg/kg food, and the maximum use levels are in the range of 1-50 mg/kg (EFFA, 2003o; EFFA, 2007a; Flavour Industry, 2005e; Flavour Industry, 2006d; Flavour Industry, 2007d).

The mTAMDI value is 1600 microgram/person/day for five of the six candidate substances from structural class I (see Section 5) as well as for the one candidate substance from structural class III. The mTAMDI value is 2100 microgram/person/day for [FL-no: 07.254] assigned to structural class I.

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

4. Absorption, Distribution, Metabolism and Elimination

The present FGE consists of seven aromatic ketones, all containing the ketone group in an aliphatic side-chain. One of the ketones contains a naphthyl group instead of a phenyl group.

No studies on absorption, distribution, metabolism or elimination were available for the seven candidate substances; however, a number of studies on supporting substances have been considered. Based on these studies, it is concluded that the candidate substances 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07:195], 3-hydroxy-4-phenylbutane-2-one [FL-no: 07.242], 2-methoxy-acetophenon [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are readily absorbed from the gut. Toxicokinetic data for a representative structurally related substance (4-phenyl-3-buten-2-one [FL-no: 07.024]) indicate that orally administered phenyl alkyl ketones undergo essentially complete first-pass metabolism prior to systemic distribution (Sauer et al., 1997b; Sauer et al., 1997a).

The phenyl-substituted alkyl ketones can be metabolised *via* reduction or oxidation and subsequent conjugation with glucuronic acid or glycine. Reduction to the corresponding secondary alcohols is either followed by conjugation with glucuronic acid and excretion, primarily in the urine, or oxidation and excretion, mainly as glycine conjugates in the urine within 24 hours. These ketones may undergo omega-oxidation in the side-chain to yield intermediary metabolites (e.g. hydroxyacetophenone) that undergo further oxidation and cleavage to yield aromatic carboxylic acids (phenylacetic acid or benzoic acid, depending on the number of carbon atoms in the side-chain). These metabolic pathways have been observed in rodent species, dogs and humans.

No information is available on the toxicokinetics of either the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214] or its supporting substance methyl 2-naphthyl ketone [FL-no: 07.013]. It could be hypothesised that the carbonyl group could be reduced and then conjugated, as described for the phenyl substituted alkyl ketones. However, the occurrence of other, possibly harmful, metabolic pathways related to the naphthalene moiety cannot be ruled out.

In conclusion, it can be anticipated that the six candidate substances 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], 3-hydroxy-4-phenylbutane-2-one [FL-no: 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are metabolised to innocuous products. This cannot be anticipated for the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214].

For more detailed information, see Annex III.

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 seven candidate substances from chemical group 21 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2.

Step 1

Six of the candidate substances, 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], 3-hydroxy-4-phenylbutane-2-one [FL-no: 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978) into structural class I, and one candidate substance, alpha-methyl naphthyl ketone [FL-no: 07.214] is classified into structural class III.

Step 2

Step 2 requires consideration of the metabolism of the candidate substances. It can be anticipated that six candidate substances, 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], 3-hydroxy-4-phenylbutane-2-one [FL-no 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are metabolised to innocuous products. Accordingly, the evaluation of these six candidate substances proceeds via the A-side of the Procedure scheme. The seventh candidate substance, alpha-methyl naphthyl ketone [FL-no: 07.214], cannot be anticipated to be metabolised to innocuous products and thus the evaluation of [FL-no: 07.214] proceeds via the B-side of the Procedure.

Step A3

The six candidate substances [FL-no: 07.193, 07.194, 07.195, 07.242, 07.254 and 07.259] have estimated European daily per capita intakes ranging from 0.0012 to 0.85 microgram (Table 2). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

Based on results of the safety evaluation sequence of the Procedure, these six candidate substances, proceeding via the A-side of the Procedure scheme, do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

Step B3

The estimated daily per capita intake of the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214] is 0.0012 microgram, which is below the threshold for its structural class of 90 microgram/person/day (class III). Accordingly, the evaluation of the substance proceeds to step B4 of the Procedure.

Step B4

For the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214] a margin of safety was estimated based upon the No Observed Adverse Effect Level (NOAEL) available for the supporting substance methyl 2-naphthyl ketone [FL-no: 07.013] of 33 mg/kg (90-day oral study in rats by Oser et al., 1965, see Section 8.2.) The MSDI value of 0.0012 microgram/capita/day is equivalent to 0.00002 microgram/kg body weight (bw)/day, at a body weight of 60 kg. Thus, the margin of safety is 2x109.

Based on results of the safety evaluation sequence of the Procedure, alpha-methyl naphthyl ketone [FL-no: 07.214], proceeding via the B-side of the Procedure scheme, does 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 intake for each of five of the six candidate substances [FL-no: 07.193, 07.194, 07.195, 07.242 and 07.259] in structural class I, based on the mTAMDI, is 1600 microgram/person/day, which is below the threshold of concern of 1800 microgram/person/day. The estimated intake for [FL-no: 07.254] in structural class I, based on the mTAMDI, is 2100 microgram/person/day, which is above the threshold of concern.

The estimated intake of the substance [FL-no: 07.214] assigned to structural class III, based on the mTAMDI, is 1600 microgram/person/day, which is above the threshold of concern for structural class III of 90 microgram/person/day.

Thus, for two candidate substances [FL-no: 07.214 and 07.254] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, see Table 6.1

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)
07.193	1-Phenylbutan-1-one	0.24	1600	Class I	1800
07.194	4-Phenylbutan-2-one	0.0012	1600	Class I	1800
07.195	1-Phenylpropan-2-one	0.85	1600	Class I	1800
07.242	3-Hydroxy-4-phenylbutane-2-one	0.016	1600	Class I	1800
07.254	2-Methoxy-acetophenon	0.37	2100	Class I	1800

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)
07.259	2-Methyl-acetophenone	0.012	1600	Class I	1800
07.214	alpha-Methyl naphthyl ketone	0.0012	1600	Class III	90

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 MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003p; Flavour Industry, 2005e; Flavour Industry, 2006d; Flavour Industry, 2007d), the combined estimated daily *per capita* intake as flavourings of the six candidate substances belonging to structural class I is 1.5 microgram. This value does not exceed the threshold of concern for structural class I of 1800 microgram/person/day.

The seven candidate substances are structurally related to 30 supporting substances evaluated by the JEFCA at its 57th meeting (JECFA, 2002b). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 26 of the 30 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 400 and 6.4 microgram/*capita*/day for structural class I and III substances, respectively, which do not exceed the thresholds of concern.

8. Toxicity

8.1. Acute Toxicity

Data are available for two of the candidate substances, 4-phenylbutan-2-one [FL-no: 07.194] and alpha-methyl naphthyl ketone [FL-no: 07.214]. The oral LD_{50} values, in mice or rats, varied from 800 mg/kg body weight (bw) up to 3200 mg/kg bw.

Thirteen of the 30 supporting substances were tested for acute toxicity in mice and/or rats. The oral LD_{50} values in mice and rats for the supporting substances range from 400 mg/kg bw to more than 5000 mg/kg bw.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute and subchronic toxicity data are available for one of the candidate substances (alpha-methyl naphthyl ketone [FL-no: 07.214]) and for nine of the 30 supporting substances of the present flavouring group.

Data on repeated dose toxicity of alpha-methyl naphthyl ketone are available from one poorly reported study on male rats (5 males/group; doses of 0, 100, 500, or 1000 mg/kg bw/day, oral, gavage). One animal treated with 1000 mg/kg died after the second dose and the remaining individuals of this treatment group were sacrificed the same day. The other rats were dosed as follows: 500 mg/kg: 12 doses (16 days); 100 mg/kg: 13 doses (17 days) (Eastman Kodak Co., 1992b). Overall, the dose of 100 mg/kg may be considered as a Lowest Observed Adverse Effect Level (LOAEL) (increase in absolute and relative liver weights, minimal hepatocyte hypertrophy, hyaline droplet formation in the kidney). The LOAEL for the candidate substance is close to a No Observed Adverse Effect Level (NOAEL) of 33 mg/kg bw established by a 90 day study on the supporting substance methyl 2-naphthyl ketone [FL-no: 07.013]. Doses of 33 and 37 mg methyl 2-naphthyl ketone/kg bw were administered via the diet to male and female rats (15/sex/group), respectively (Oser et al., 1965). No effects on growth, food consumption, haematology, blood chemistry, liver and kidney weights or on gross and microscopic appearance of major organs were found. In conclusion, the NOAEL of 33 mg/kg bw for methyl 2-naphthyl ketone [FL-no: 07.013] is used to estimate a margin of safety for the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214].

No chronic or carcinogenicity studies are available for the candidate substances. Carcinogenicity studies are available for one supporting substance, 1-phenylethan-1-ol [FL-no: 02.064] performed in mice and rats (NTP, 1990d). These studies were evaluated by the JECFA at its 41st meeting (JECFA, 1993b) when the JECFA reviewed a series of studies on 1-phenylethan-1-ol (alpha-methylbenzyl alcohol), and concluded: "The Committee noted that *alpha*-methylbenzyl alcohol administered by gavage in corn oil was associated with a higher incidence of renal tubule-cell adenomas in male rats than in untreated controls, but not in female rats or in mice, at dose levels at or exceeding the maximum tolerated dose (MTD) and in the presence of factors that exacerbated a high incidence of age-related chronic progressive nephropathy. The intake of this compound from all sources is extremely low. On the basis of the evidence available, the Committee concluded that the higher incidence of benign neoplasms in the kidney of male rats is not relevant for humans. In view of the limited database, the Committee concluded that the available data could be used to set an Acceptable Daily Intake (ADI) by application of a safety factor of 1000 to the minimal effect level of 93 mg/kg of body weight per day with respect to liver weight increase in the absence of associated pathology in the 13-week study in rats. Accordingly, an ADI of 0-0.1 mg/kg of body weight per day was allocated for *alpha*-methylbenzyl alcohol." The Panel concurred with this conclusion.

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data on developmental toxicity and reproductive toxicity have been published for one of the candidate substances, alpha-methyl naphthyl ketone [FL-no: 07.214] (Sporn et al., 1963), but this study is considered to be of insufficient quality.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

In vitro data are only available for two candidate substances, which were negative in tests for mutagenicity in bacteria, however, these studies are of insufficient quality. All other *in vitro* data are related to nine supporting substances. For these nine substances tested in the Ames test the results were negative. However, two of the supporting substances, 1-phenylethan-1-ol [FL-no: 02.064] and acetophenone [FL-no: 07.004] induced chromosomal aberrations *in vitro* in the presence of metabolic activation, and 1-phenylethan-1-ol was also weakly positive in the mouse lymphoma *tk* assay.

There are negative *in vivo* micronucleus tests on two supporting substances (4-(4-methoxyphenyl)-2-butanone [FL-no: 07.029] and methyl-2-naphthyl ketone [FL-no: 07.013]), but these studies are not considered valid.

Overall, the limited data available do not allow a final assessment of genotoxicity. From the data available there is some indication of genotoxic potential for two of the supporting substances (1-phenylethan-1-ol and acetophenone). However, in the light that 1-phenylethan-1-ol can be metabolised to acetophenone and *vice vers*a and that the results of a carcinogenicity study with 1-phenylethan-1-ol in mice and rats do not give rise to concern, the Panel concluded that the positive *in vitro* results for the two supporting substances do not give rise to concern with respect to carcinogenicity in humans. Regarding the prediction of risk of heritable mutations to man, neither adequate data on germ cell mutagenicity were available nor data from a two-generation developmental toxicity study. However, toxicokinetic data (Sauer et al., 1997a; Sauer et al., 1997b) for another structurally related substance (4-phenyl-3-buten-2-one [FL-no: 07.024]) indicate that orally administered phenyl alkyl ketones undergo essentially complete first-pass metabolism prior to systemic distribution and that germ cells are unlikely to be exposed. Therefore, the Panel concluded that the positive *in vitro* results for the two supporting substances do likewise not give rise to concern with respect to heritable mutations in humans and that, finally, the positive *in vitro* results for the two supporting substances do not preclude their evaluation through the Procedure.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The present Revision of FGE.16, FGE.16Rev2, includes the assessment of one additional candidate substance [FL-no: 07.252], compared to FGE.16Rev1.

In total FGE16.Rev2 deals with seven candidate substances, which are aromatic ketones belonging to chemical group 21.

One of the seven candidate substances can exist as an optical isomer [FL-no: 07.242].

Six of the flavouring substances are classified into structural class I and one substance is classified into structural class III.

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

According to the default MSDI approach, the seven flavouring substances in this group have intakes in Europe from 0.0012 to 0.85 microgram/*capita*/day, which are below the threshold of concern values for structural class I of 1800 microgram/person/day and for structural class III of 90 microgram/person/day.

On the basis of the reported annual production in Europe (MSDI approach) the combined intake of the six candidate substances belonging to structural class I would result in an intake of approximately 1.5 microgram/*capita*/day. This value is lower than the threshold of concern for a structural class I substance. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 26 of the 30 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 400 and 6.4 microgram/*capita*/day for structural class I and III, respectively, which do not exceed the thresholds of concern.

Overall, the limited data available do not allow a final assessment of genotoxicity. From the data available there is some indication of genotoxic potential for two of the supporting substances (1-phenylethan-1-ol and acetophenone). However, taking into consideration metabolism and

carcinogenicity data on the candidate and supporting substances, the positive *in vitro* results do not preclude their evaluation through the Procedure.

It can be anticipated that the six candidate substances 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], 3-hydroxy-4-phenylbutan-2-one [FL-no: 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are metabolised to innocuous products at the estimated levels of intake. This cannot be anticipated for alpha-methyl naphthyl ketone [FL-no: 07.214].

Based on a NOAEL of 33 mg/kg bw/day for the supporting substance methyl 2-naphthyl ketone [FL-no: 07.013], which is structurally related to the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214] a margin of safety of approximately $2x10^9$ could be estimated for this candidate substance. Thus alpha-methyl naphthyl ketone [FL-no: 07.214] is not expected to be of safety concern at its estimated level of intake as flavouring substance.

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

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

When the estimated intakes were based on the mTAMDI approach they were 1600 microgram/person/day for five of the six flavouring substances belonging to structural class I and 2100 microgram/person/day for [FL-no: 07.254]. Thus, the intakes were below the threshold of concern for structural class I of 1800 microgram/person/day for five of the six candidate substances belonging to structural class I. For the flavouring substance alpha-methyl naphthyl ketone [FL-no: 07.214] belonging to structural class III the intake was above the threshold of concern of 90 microgram/person/day for structural class III. The five substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

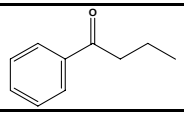
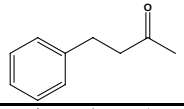
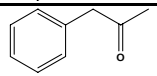
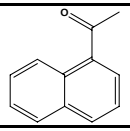
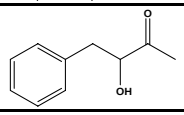
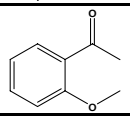
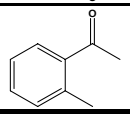
Thus, for two of the seven flavouring substances considered in this Opinion the intake, estimated on the basis of the mTAMDI, exceeds the relevant threshold for its structural class, to which the flavouring substance has been assigned. Therefore, for alpha-methyl naphthyl ketone [FL-no: 07.214] and for 2-methoxy-acetophenone [FL-no: 07.254] more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the seven candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity have been provided for the six of the seven materials of commerce. For one candidate substance [FL-no: 07.242], the optical isomerism has not been specified. Thus, the final evaluation of the material of commerce cannot be performed for this substance, pending further information on chirality.

The remaining six substances [FL-no: 07.193, 07.194, 07.195, 07.214, 07.254 and 07.259] 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 FGE.16REV2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 16, Revision 2

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
07.193	1-Phenylbutan-1-one		 495-40-9	Liquid $C_{10}H_{12}O$ 148.20	Practically insoluble or insoluble 1 ml in 1 ml	229 MS 95 %	1.517-1.523 0.986-0.992	
07.194	4-Phenylbutan-2-one		 11182 2550-26-7	Liquid $C_{10}H_{12}O$ 148.20	Practically insoluble or insoluble 1 ml in 1 ml	235 MS 99 %	1.509-1.515 0.985-0.991	
07.195	1-Phenylpropan-2-one		 11042 103-79-7	Liquid $C_9H_{10}O$ 134.18	Practically insoluble or insoluble 1 ml in 1 ml	214 MS 95 %	1.513-1.519 1.004-1.010	
07.214	alpha-Methyl naphthyl ketone		 941-98-0	Liquid $C_{12}H_{10}O$ 170.21	Practically insoluble or insoluble 1 ml in 1 ml	298 10 MS 95 %	1.625-1.631 1.116-1.122	
07.242	3-Hydroxy-4-phenylbutane-2-one 6)		4052 5355-63-5	Liquid $C_{10}H_{12}O_2$ 164.2	Practically insoluble or insoluble 1 ml in 1 ml	98 (0.1 hPa) NMR MS 93 %	1.526-1.532 1.080-1.086	
07.254	2-Methoxy-acetophenon		4163 579-74-8	Liquid $C_9H_{10}O_2$ 150.18	Soluble Soluble	245 MS 99 %	1.536-1.542 1.088-1.092	Register name to be changed to 2-Methoxy-acetophenone.
07.259	2-Methyl-acetophenone		4316 557-16-2	Liquid $C_9H_{10}O$ 134.18	Practically insoluble or insoluble 1 ml in 1 ml	214 IR NMR MS > 97 %	1.526-1.532 1.023-1.029	CASrn not valid - to be replaced by 577-16-2.

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.

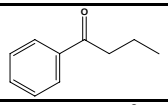
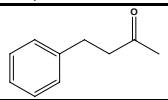
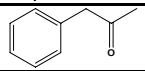
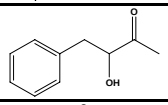
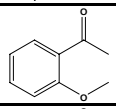
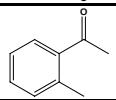
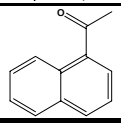
4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

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

Table 2: 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
07.193	1-Phenylbutan-1-one		0.24	Class I A3: Intake below threshold	4)	6)	
07.194	4-Phenylbutan-2-one		0.0012	Class I A3: Intake below threshold	4)	6)	
07.195	1-Phenylpropan-2-one		0.85	Class I A3: Intake below threshold	4)	6)	
07.242	3-Hydroxy-4-phenylbutane-2-one		0.016	Class I A3: Intake below threshold	4)	7)	
07.254	2-Methoxy-acetophenon		0.37	Class I A3: Intake below threshold	4)	6)	a)
07.259	2-Methyl-acetophenone		0.012	Class I A3: Intake below threshold	4)	6)	
07.214	alpha-Methyl naphthyl ketone		0.0012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

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

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

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

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

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

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

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

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

a) *Register name to be changed to 2-Methoxy-acetophenone.*

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

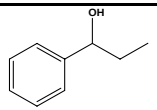
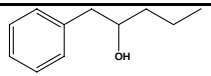
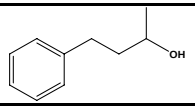
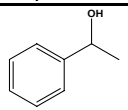
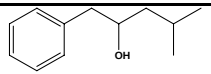
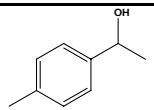
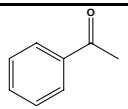
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.033	1-Phenylpropan-1-ol		2884 82 93-54-9	822 JECFA specification (JECFA, 2001c)	0.24	No safety concern a) Category B b)	The JECFA evaluated 1-phenyl-1-propanol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
02.034	1-Phenylpentan-2-ol		2953 83 705-73-7	825 JECFA specification (JECFA, 2001c)	0.12	No safety concern a) Category B b)	The JECFA evaluated alpha-propylphenethyl alcohol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
02.036	4-Phenylbutan-2-ol		2879 85 2344-70-9	815 JECFA specification (JECFA, 2001c)	1.2	No safety concern a) Category B b)	
02.064	1-Phenylethan-1-ol		2685 2030 98-85-1	799 JECFA specification (JECFA, 2001c)	27	No safety concern a) Deleted b)	ADI: 0-0.1 (JECFA, 1993a). The JECFA evaluated alpha-methylbenzyl alcohol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
02.065	4-Methyl-1-phenylpentan-2-ol		2208 2031 7779-78-4	827 JECFA specification (JECFA, 2001c)	24	No safety concern a) Category A b)	The JECFA evaluated alpha-isobutylphenethyl alcohol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
02.080	1-(p-Tolyl)ethan-1-ol		3139 10197 536-50-5	805 JECFA specification (JECFA, 2001c)	0.12	No safety concern a)	The JECFA evaluated p,alpha-dimethylbenzyl alcohol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
07.004	Acetophenone		2009 138 98-86-2	806 JECFA specification (JECFA, 2001c)	15	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

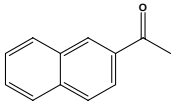
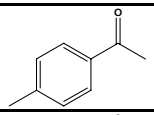
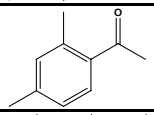
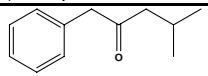
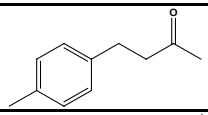
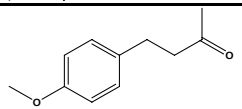
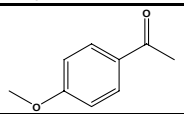
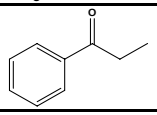
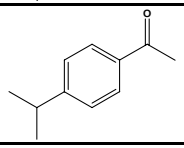
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.013	Methyl 2-naphthyl ketone		2723 147 93-08-3	811 JECFA specification (JECFA, 2001c)	6.3	No safety concern a) Category A b)	No ADI allocated (JECFA, 1981a). The JECFA evaluated methyl beta-naphthyl ketone (CASrn as in Register).
07.022	4-Methylacetophenone		2677 156 122-00-9	807 JECFA specification (JECFA, 2001c)	22	No safety concern a) Category B b)	
07.023	2,4-Dimethylacetophenone		2387 157 89-74-7	809 JECFA specification (JECFA, 2001c)	0.24	No safety concern a) Category B b)	
07.025	4-Methyl-1-phenylpentan-2-one		2740 159 5349-62-2	828 JECFA specification (JECFA, 2001c)	8.5	No safety concern a) Category B b)	The JECFA evaluated 4-methyl-1-phenyl-2-pentanone (CASrn as in Register).
07.026	4-(p-Tolyl)butan-2-one		3074 160 7774-79-0	817 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Category B b)	The JECFA evaluated 4-(p-totyl)-2-butanone (CASrn as in Register).
07.029	4-(4-Methoxyphenyl)butan-2-one		2672 163 104-20-1	818 JECFA specification (JECFA, 2001c)	4.5	No safety concern a) Category A b)	The JECFA evaluated 4-(p-methoxyphenyl)-2-butanone (CASrn as in Register).
07.038	4-Methoxyacetophenone		2005 570 100-06-1	810 JECFA specification (JECFA, 2001c)	130	No safety concern a) Category B b)	The JECFA evaluated acetanisole (CASrn as in Register).
07.040	1-Phenylpropan-1-one		3469 599 93-55-0	824 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Category A b)	The JECFA evaluated propiophenone (CASrn as in Register).
07.042	4-Isopropylacetophenone		2927 651 645-13-6	808 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Category B b)	The JECFA evaluated p-isopropylacetophenone (CASrn as in Register).

Table 3: Supporting Substances Summary

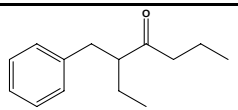
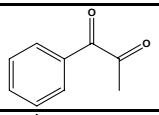
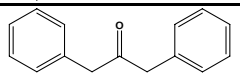
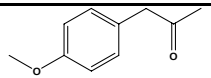
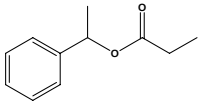
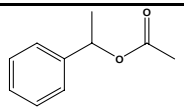
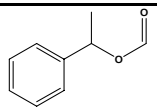
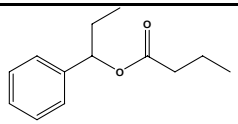
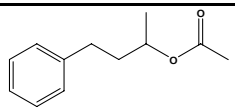
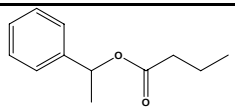
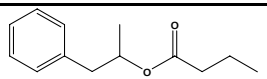
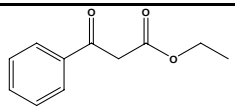
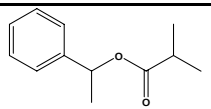
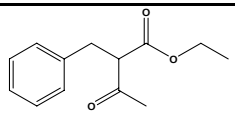
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.070	3-Benzylheptan-4-one		2146 2140 7492-37-7	830 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Deleted b)	The JECFA evaluated 3-benzyl-4-heptanone (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
07.079	1-Phenylpropan-1,2-dione		3226 2275 579-07-7	833 JECFA specification (JECFA, 2001c)	4.9	No safety concern a) Category B b)	The JECFA evaluated 1-phenyl-1,2-propanedione (CASrn as in Register).
07.086	1,3-Diphenylpropan-2-one		2397 11839 102-04-5	832 JECFA specification (JECFA, 2001c)	0.12	No safety concern a)	The JECFA evaluated 1,3-diphenyl-2-propanone (CASrn as in Register).
07.087	4-Methoxyphenylacetone		2674 11836 122-84-9	813 JECFA specification (JECFA, 2001c)	0.12	No safety concern a)	The JECFA evaluated 1-(p-methoxyphenyl)-2-propanone (CASrn as in Register).
09.144	1-Phenethyl propionate		2689 425 120-45-6	802 JECFA specification (JECFA, 2001c)	0.97	No safety concern a) Category B b)	The JECFA evaluated alpha-methylbenzyl propionate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.178	1-Phenethyl acetate		2684 573 93-92-5	801 JECFA specification (JECFA, 2001c)	170	No safety concern a) Category A b)	The JECFA evaluated alpha-methylbenzyl acetate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.179	1-Phenethyl formate		2688 574 7775-38-4	800 JECFA specification (JECFA, 2001c)	0.037	No safety concern a) Category B b)	The JECFA evaluated alpha-methylbenzyl formate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.189	1-Phenylpropyl butyrate		2424 628 10031-86-4	823 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Category B b)	The JECFA evaluated alpha-ethylbenzyl butyrate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.

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
09.200	1-Methyl-3-phenylpropyl acetate		2882 671 10415-88-0	816 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Category B b)	The JECFA evaluated 4-phenyl-2-butyl acetate (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
09.231	1-Phenethyl butyrate		2686 2083 3460-44-4	803 JECFA specification (JECFA, 2001c)	1.1	No safety concern a) Category B b)	The JECFA evaluated alpha-methylbenzyl butyrate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.249	1-Methyl-2-phenethyl butyrate		3197 2276 68922-11-2	814 JECFA specification (JECFA, 2002d)	0.12	No safety concern a) Category B b)	The JECFA evaluated alpha-methylphenethyl butyrate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.476	Ethyl 3-phenyl-3-oxopropionate		2423 627 94-02-0	834 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Category B b)	The JECFA evaluated ethyl benzoylacetate (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
09.486	1-Phenethyl isobutyrate		2687 2088 7775-39-5	804 JECFA specification (JECFA, 2001c)	24	No safety concern a) Category B b)	The JECFA evaluated alpha-methylbenzyl isobutyrate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.501	Ethyl 2-acetyl-3-phenylpropionate		2416 2241 620-79-1	835 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Category B b)	The JECFA evaluated ethyl 2-acetyl-3-phenylpropionate (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.

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, 2002b).

b) (CoE, 1992).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), 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.

[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).

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

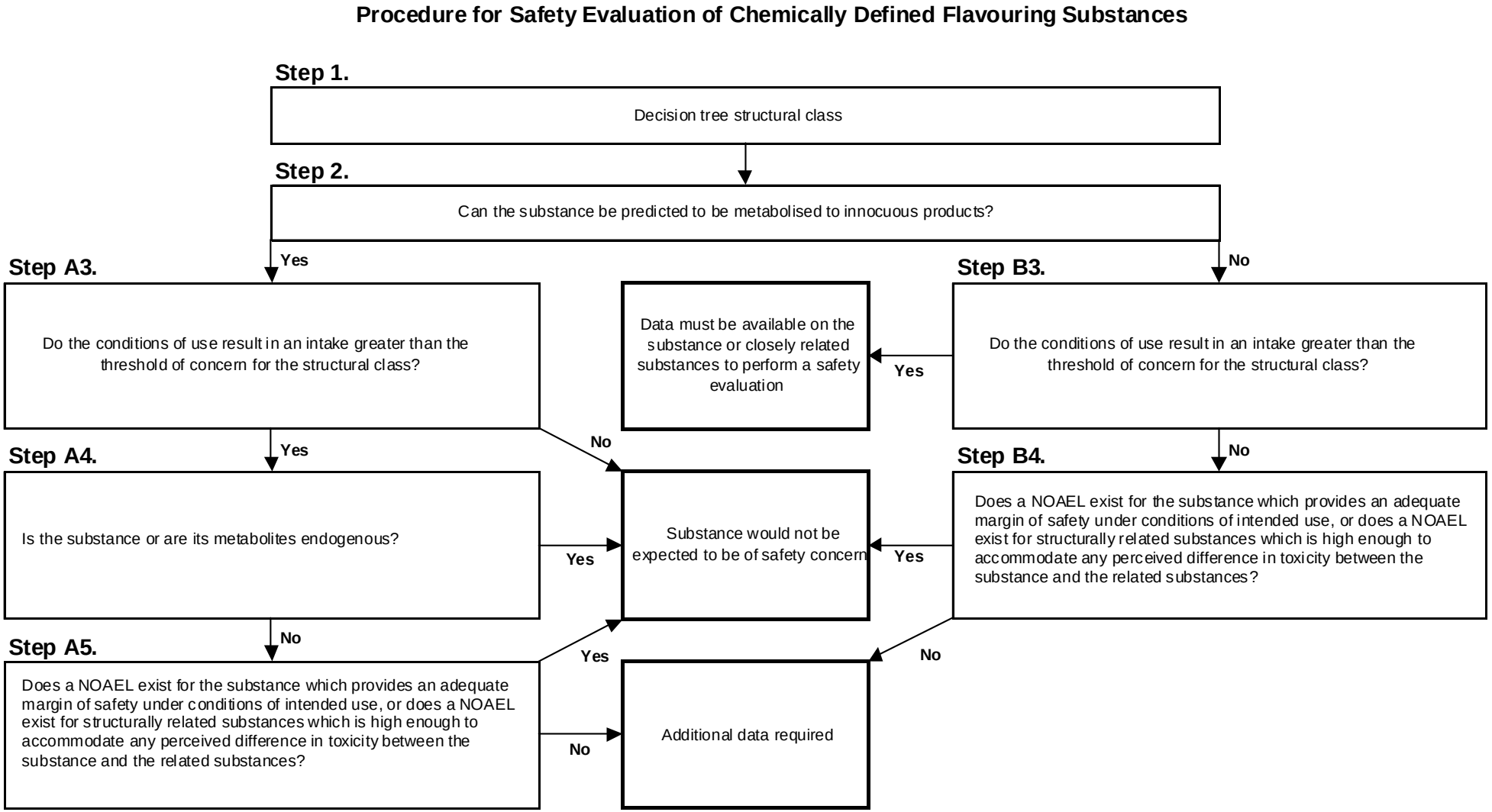


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a). 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, 2000a)

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

Table II.1.2.Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.16rev2 (EFFA, 2003o; EFFA, 2007a; Flavour Industry, 2005e; Flavour Industry, 2006d; Flavour Industry, 2007d).

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
07.193	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.194	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.195	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.214	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.242	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.254	5	10	1	-	-	5	-	10	-	-	-	-	1	-	1	3	10	-
	20	20	10	-	-	20	-	50	-	-	-	-	10	-	10	30	20	-
07.259	3	2	3	2	2	4	2	5	1	1	-	-	2	3	2	4	5	2
	5	10	15	10	10	20	10	25	5	5	-	-	10	1	10	20	25	10

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 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, 2000a) 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, 2000a)
- 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, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- 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, 2000a) 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
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	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		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) 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	
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food	
10.0	Eggs and egg products	Food	
11.0	Sweeteners, including honey		Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.		Exception d
13.0	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
15.0	Ready-to-eat savouries		Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food	

The mTAMDI values (see Table II.2.3) are presented for each of the seven flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003o; EFFA, 2007a; Flavour Industry, 2005e; Flavour Industry, 2006d; Flavour Industry, 2007d). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
07.193	1-Phenylbutan-1-one	1600	Class I	1800
07.194	4-Phenylbutan-2-one	1600	Class I	1800
07.195	1-Phenylpropan-2-one	1600	Class I	1800
07.242	3-Hydroxy-4-phenylbutane-2-one	1600	Class I	1800
07.254	2-Methoxy-acetophenone	2100	Class I	1800
07.259	2-Methyl-acetophenone	1600	Class I	1800
07.214	alpha-Methyl naphthyl ketone	1600	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The present FGE consists of seven aromatic ketones, all containing the ketone group in an aliphatic side-chain. One of the ketones contains a naphthyl group instead of a phenyl group.

No studies on absorption, distribution, metabolism or elimination were available for the seven candidate substances. However, a number of studies on supporting substances have been found and are reported in the following.

III.2. Absorption, Distribution and Elimination

Acetophenone [FL-no: 07.004], 1-phenylethan-1-ol [FL-no: 02.064], and other structurally related aromatic ketones and alcohols, have been shown to be rapidly absorbed from the gut, metabolised in the liver and excreted primarily in the urine, and to a very minor extent, in the faeces. Toxicokinetic data on a structurally related substance trans-4-phenyl-3-buten-2-one [FL-no: 07.024] suggest that oral doses of this ketone undergo essentially complete first-pass hepatic clearance in both mice and rats. The observation that the systemic clearance of the ketone is approximately equivalent to its rate of absorption, together with the absence or very low levels of ketone in systemic blood after oral dosing, allow to conclude that tissue exposure to the parent compound is expected to be extremely limited (Sauer et al., 1997a; Sauer et al., 1997b).

Early studies showed that acetophenone [FL-no: 07.004] or 1-phenylethan-1-ol [FL-no: 02.064] (Quick, 1928a; Smith et al., 1954a; Thierfelder & Daiber, 1923) as well as other ketones derived from alkyl benzenes, namely ethyl phenyl, propyl phenyl, methyl benzyl, ethyl benzyl and methyl phenethyl ketones (Smith et al., 1954c) are absorbed, metabolised and excreted as polar metabolites within 24 hours.

Approximately half of the 450 mg/kg bw oral dose of acetophenone [FL-no: 07.004] or 460 mg/kg bw dose of 1-phenylethan-1-ol [FL-no: 02.064] fed to rabbits were present in the urine after 24 hours (Smith et al., 1954a). Similarly, approximately half of a 500 mg/kg bw dose of acetophenone administered to dogs in the diet was recovered as polar metabolites in urine samples within 24 hours (Quick, 1928a).

In a toxicokinetic study (Sauer et al., 1997a), male F344 rats (3/group) were given single oral doses of 200 mg/kg bw of 14C-ring labelled 4-phenyl-3-buten-2-one [FL-no: 07.024] by gavage. Periodic collection of urine (6, 12, 24 and 48 hours), faeces (24 and 48 hours), blood (0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 12, 24 and 48 hours after dosing) and tissues (48 hours) revealed that >70 % of the radiolabel was excreted in the urine within six hours and >96.6 % within 48 hours. After 48 hours, only 4.8 % of radioactivity was measured in the faeces, while <0.2 % was retained in the tissue. No parent ketone could be detected in the blood at any time during the experiment. For comparison, 20 mg/kg bw of the radiolabelled ketone was intravenously administered to rats: a strikingly similar pattern of absorption and excretion was obtained. Blood concentrations of the ketone were below limits of detection after 60 minutes, and 48 hours after dosing essentially 100 % of the radioactivity was accounted for in the urine and faeces. The volume of distribution was relatively small, suggesting that only a limited amount of the dose is distributed to the tissues. The high systemic clearance (69.8 ml/kg/min) approximately equivalent to hepatic clearance, suggests that the ketone undergoes essentially complete first-pass hepatic clearance. Small quantities of intact compound (0.8 %-1.6 %) were detected in the urine after oral administration, suggesting that a small percentage of the compound is absorbed intact and escapes metabolism (Sauer et al., 1997a).

In a parallel study (Sauer et al., 1997b), female B6C3F$_1$ mice (3/group) were given single oral doses of 200 mg/kg bw of 14C-ring labelled 4-phenyl-3-buten-2-one [FL-no: 07.024] by gavage, following the protocol described above. Greater than 84 % of the radiolabel was excreted in the urine within six hours and >94 % within 48 hours. After 48 hours, 1.2 % of radioactivity was measured in the faeces and 0.3 % in exhaled air. Unlike in rats, the parent ketone was detected in the blood, although it accounted for only 2.6 % of the total dose. Following intravenous administration (20 mg/kg bw), blood ketone levels were below limits of detection after 30 minutes. The disposition half-life (8.9 min), volume of distribution (3.3 litres/kg bw), and high systemic clearance (540 ml/ min/kg) exceeding both hepatic blood flow (ca.110 ml/min/kg) and cardiac output (ca. 400 ml/min/kg), indicate that

1) the ketone was cleared more rapidly from the blood of mice than that of rats

2) a very efficient extra-hepatic clearing mechanism occurs and

3) the parent ketone appeared to be significantly distributed to different tissues. The appearance of the parent ketone in the blood of mice could be due to the higher rate of intestinal absorption compared to that of rats (Sauer et al., 1997b).

Based on these studies, it may be concluded that aryl ketones are rapidly absorbed, efficiently metabolised in the liver, and excreted mainly in the urine within 24 hours.

III.3. Metabolism

Acetophenone [FL-no: 07.004] and 1-phenylethan-1-ol [FL-no: 02.064] are readily interconvertible and show similar excretion patterns. Reduction of acetophenone to 1-phenylethan-1-ol and oxidation of 1-phenylethan-1-ol to acetophenone have been reported to occur in rat hepatic subcellular fractions such as cytosol and microsomal preparations in the presence of NAD+ and NADP+ (Hopkins et al., 1972; Maylin et al., 1973). The oxidation reaction in microsomes is catalysed by two distinct enzymes; the major contribution to the activity is given by the P450 system, as indicated by the inhibition of the oxidase activity by carbon monoxide, its dependence on NADPH and induction by phenobarbital pretreatment (Maylin et al., 1973). In the soluble fraction there are at least two distinct dehydrogenase activities able to oxidise 1-phenylethan-1-ol to acetophenone: one is strictly dependent on the presence of NAD+ and active on (+) and (-)1-phenylethan-1-ol whereas the NADP+-dependent system is specific for the reaction involving the (-) isomer (Callaghan et al., 1973). The reduction and oxidation steps have been shown to be stereoselective both in vitro and in vivo (Culp & McMahon, 1968; Callaghan et al., 1973; Sullivan et al., 1976). The alcohol is mainly conjugated with glucuronic acid and excreted, whereas the ketone undergoes omega-oxidation and subsequent oxidative decarboxylation to yield benzoic acid that is excreted mainly in the urine as hippuric acid. Little or no oxidation of the aromatic ring has been observed.

The reduction and oxidation pathways have been observed in animal species other than rats. *In vitro* incubation of acetophenone [FL-no: 07.004] with carbonyl reductase from rabbit kidney resulted in the predominant formation of S-(-)-1-phenylethan-1-ol (Culp & McMahon, 1968). *In vivo* acetophenone administered to rabbits via different routes or to dogs in the diet is primarily reduced to 1-phenylethan-1-ol. When dogs were administered acetophenone as single oral doses of 500 mg/kg bw, 35 % was recovered in the urine as the glucuronic acid conjugate of 1-phenylethan-1-ol, whereas 20 % was excreted as hippuric acid. Much of the remainder was excreted unchanged (Quick, 1928a).

When acetophenone [FL-no: 07.004] was administered in single subcutaneous doses of 500 to 1400 mg/kg bw to dogs, the major urinary metabolites were the glucuronic acid conjugate of 1-phenylethan-1-ol (35 %) and hippuric acid (24 %). Small amounts were excreted as mandelic acid or unchanged (Thierfelder & Daiber, 1923).

A 450 mg/kg bw oral dose of acetophenone was excreted in the 24-hour urine, 47 % as the glucuronic acid conjugate of 1-phenylethan-1-ol and 17 % as hippuric acid (Smith et al., 1954c).

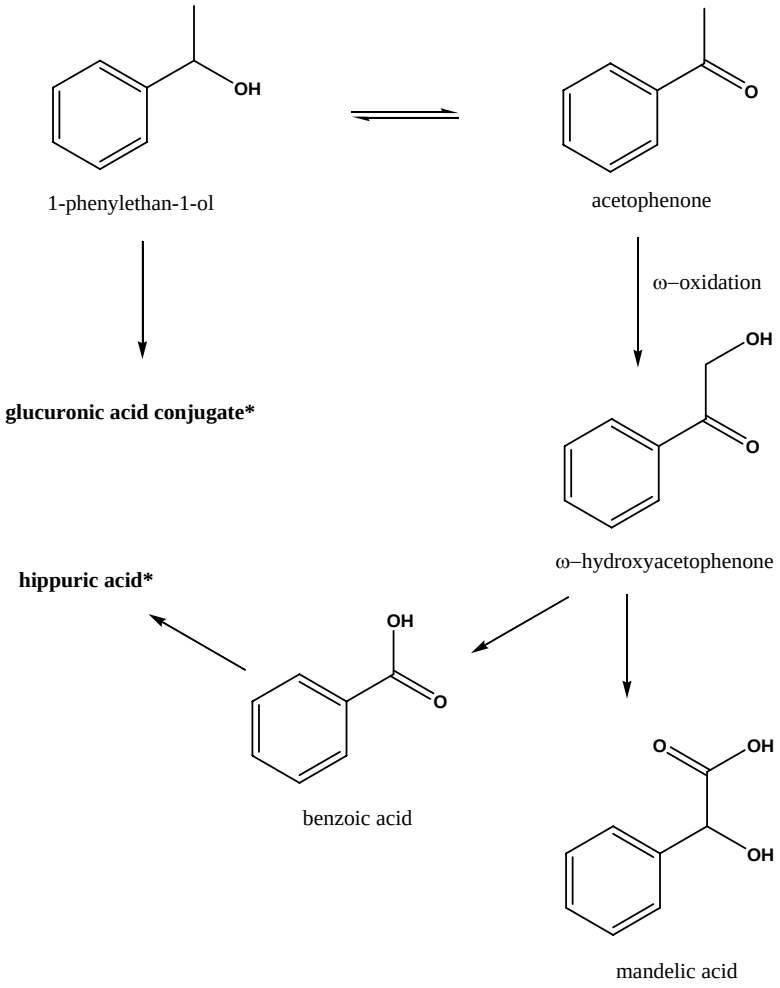
When rabbits were given large doses (5.36 g total dose) of acetophenone by intraperitoneal (i.p.) injection in addition to 1-phenylethan-1-ol and its glucuronide, hippuric acid and mandelic acid minor urinary metabolites were detected including omega-hydroxyacetophenone, m-hydroxy-acetophenone and p-hydroxyacetophenone. These minor metabolites accounted for approximately 1 % of the dose (Kiese & Lenk, 1974).

Taking into account that the major metabolite of ethylbenzene is 1-phenylethan-1-ol, studies related to this compound may be considered relevant to provide information also on acetophenone metabolism. In rabbits, 60-70 % of a single 244 mg/kg bw dose of ethyl benzene administered via stomach tube undergoes alpha-oxidation to yield 1-phenylethan-1-ol, which is recovered in the urine as glucuronide and hippuric acid within 24 hours; 10-20 % undergoes omega-oxidation followed by glycine conjugation (El Masry et al., 1956).

In vivo studies in rats dosed intraperitoneally (i.p.) with ethyl benzene, 1-phenylethan-1-ol, acetophenone, and omega-hydroxyacetophenone suggest that 1) chiral mandelic acid forms from alpha-methylbenzyl alcohol via acetophenone, 2) benzoic acid also forms directly from acetophenone, and 3) omega-hydroxyacetophenone is an intermediary metabolite in the formation of chiral mandelic acid and benzoic acid from acetophenone or 1-phenylethan-1-ol (Sullivan et al., 1976). When rats were given single i.p. doses of racemic labeled [3H-C1-1]-phenylethan-1-ol, only (-)-mandelic acid not containing any [3H-]-label was detected in the urine. This result suggests that the alcohol was oxidised to acetophenone, which has been shown to be the precursor of optically active mandelic acid. The hypothesis that omega-hydroxyacetophenone is an intermediate in the formation of benzoic acid and mandelic acid is supported by the observation that incubation of acetophenone in microsomes of rat hepatocytes yields mainly omega-hydroxyacetophenone (Sullivan et al., 1976).

It has been reported that after human exposure to ethylbenzene the metabolites mandelic acid, 1-phenylethanol and phenylglyoxylic acid have been found in the urine (Bardodej & Bardodejova, 1970); furthermore after oral doses of omega-hydroxyacetophenone human excreted mandelic and hippuric acid in the urine (Logemann et al., 1964).

Based on these observations it is concluded that, in animals, 1-phenylethan-1-ol [FL-no: 02.064] and acetophenone [FL-no: 07.004] are interconvertible. 1-Phenylethan-1-ol may be excreted in the urine predominantly as the glucuronic acid conjugate. Acetophenone undergoes omega-oxidation to yield omega-hydroxyacetophenone. Subsequent stereoselective reduction of the ketone function and oxidation of the terminal alcohol yields mandelic acid, whereas simple oxidation of the terminal alcohol yields the corresponding ketoacid which may undergo oxidative decarboxylation to yield benzoic acid, which is excreted as hippuric acid (see Figure III.1).



***Principal urinary metabolites in animals**

Figure III.1 Metabolism of acetophenone and 1-phenylethan-1-ol.

An increase of chain length or the presence of unsaturation sites do not significantly alter the metabolic fate of phenyl alkyl ketones or related alcohols. The ketones may hereby be biotransformed to the corresponding alcohols and the secondary alcohols to the ketones. In major metabolic pathways, the ketone is stereoselectively reduced to the corresponding alcohol, which is subsequently excreted as the glucuronic acid conjugate. Beta-unsaturated ketones are commonly metabolised to either benzoic acid or phenylacetic acid according to the number of carbon atoms associated with their alkene side-chains. If the alkyl chain is even numbered, the ketone may undergo oxidation and cleavage to yield phenylacetic acid or, if the alkyl chain is odd numbered, oxidative cleavage yields mainly benzoic acid. The acids are excreted almost exclusively as glycine conjugates (i.e. phenylaceturic acid and hippuric acid).

In vitro metabolisms of 1-phenyl-1-propanone [FL-no: 07.040] and 1-phenyl-2-propanone were studied in NADPH and NADH-fortified male rat and rabbit liver preparations (Coutts et al., 1981). Reduction to the corresponding alcohols was found as the major metabolic route, although aliphatic C-hydroxylation and alcohol dehydrogenation also occurred. Propiophenone produced 19-24 % and 61-75 % 1-phenyl-1-propanol with rat and rabbit liver preparations, respectively. Similarly when 1-phenyl-2-propanone was incubated with male rat and rabbit liver homogenate extensive reduction to 1-phenyl-2-propanol occurred in the presence of both cofactors. Other significant metabolic pathways detected included aliphatic hydroxylation of propiophenone to produce 1-hydroxy-1-phenyl-2-propanone and oxidation of 1-phenyl-1-propanol to

propiophenone (Coutts et al., 1981). In a follow-up experiment, the reduction of propiophenone [FL-no: 07.040], 1-phenyl-2-propanone and 1-phenylpropan-1,2-dione [FL-no: 07.079] *in vitro* and *in vivo* in rats and rabbits revealed that the corresponding alcohols were produced as a mixture of enantiomers. The mixture contains at least 70 % of the S-(-)-isomer. The highest degree of stereospecificity was shown by the reduction of propiophenone in vitro in rat and rabbit liver preparations, producing 93-97 % of the (S)-(-)-isomer of 1-phenyl-1-propanol (Prelusky et al., 1982).

When a single dose of 200 mg/kg bw of 4-phenyl-3-buten-2-one [FL-no: 07.024] was administered to male F344 rats by gavage, the glycine conjugate of phenylacetic acid was the major urinary metabolite (65 % of the dose) collected within 48 hours. Other urinary metabolites included hippuric acid (9.9 %) and glutathione conjugates of the parent ketone (5.6 %) and its related alcohol (2.2 %), but their formation is related to the presence of the double-bond in the side chain, and therefore is not relevant for the candidate substances included in the present FGE, characterised by saturated aliphatic chains. Indeed, the formation of glutathione (GSH) conjugates is due to the direct attack of GSH to the double-bond occurring in the liver; as a consequence the oral administration of the parent compound resulted in about 35 % depletion of hepatic gluthathione. Hippuric acid (benzoylglycine) is expected to be formed from hydration of the double-bond, subsequent retro-aldol reaction to form benzaldehyde, and then oxidation to benzoic acid. No glucuronide nor sulphate conjugate were detected in the urine (Sauer et al., 1997a).

In a similar experiment in female $B6C3F_1$ mice, 4-phenyl-3-buten-2-one [FL-no: 07.024] appears to be metabolised via reduction, oxidation and conjugation of the ketone group as well as at unsaturation site of the side chain (not relevant for the candidate substances). Urinary metabolites included the glycine conjugates of phenylacetic acid (35.1 %) and benzoic acid (19 %), the glutathione conjugate at the double-bond site in the side-chain (6.7 %) and the unchanged ketone (8.6 %). The principal blood metabolite after intravenous administration of the same dose was the corresponding alcohol and 4-hydroxy-4-phenyl-2-butanone due to hydration of the double bond, supporting the conclusion that the test compound can be simultaneously reduced or oxidised following administration. Only about 1.2 % of the administered dose was present in the faeces. Compared to the rat, the mouse produced approximately 2-fold more benzyl alcohol, which is converted into benzoic acid and then conjugated with glycine (Sauer et al., 1997b).

Trans-4-phenyl-3-buten-2-one [FL-no: 07.024] was also demonstrated to be biotransformed by rat liver microsomes, but not by liver cytosol, mainly to the (R)-enantiomer of trans-4-phenyl-3-buten-2-ol in the presence of NADH or NADPH. Rat blood also exhibited the carbonyl reductase activity in the presence of NADH or NADPH, although to a lesser extent (Okamoto et al., 1999). In addition to trans-4-phenyl-3-buten-2-ol, trans-4-phenyl-3-buten-2-one administered to rats and dogs intravenously at 25 mg/kg bw is metabolised to the candidate substance 4-phenylbutan-2-one [FL-no: 07.194] via reduction of the double-bond (Kitamura et al., 1999).

A study on the fate of n–propyl and n-butyl benzene in the rabbit evidenced that about 50 % of propyl benzene is excreted as the glucuronides of 1-phenylpropan-1-ol and benzylmethylcarbinol and about 15 % as hippuric acid, whose major precursor appears to be benzylmethylcarbinol. About 50 % of butylbenzene is excreted as the glucuronides of methyl-2-phenylethylcarbinol and phenylpropylcarbinol and about 20 % as phenaceturic acid, whose major precursor appears to be methyl-2-phenylethylcarbinol (El Masry et al., 1956).

The majority (46-61 %) of a single dose (364 mg/kg bw) of benzophenone [FL-no: 07.032] administered to rabbits via stomach tube was excreted as the glucuronide conjugate of the corresponding alcohol benzhydrol (Robinson, 1958). Incubation of 8 mM benzophenone with rabbit liver homogenate and NADPH resulted in the formation of 20 % benzhydrol in one hour (Leibman, 1971).

No information is available on the toxicokinetics of either the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214] or its supporting substance methyl 2-naphthyl ketone [FL-no: 07.013]. It could be hypothesised that the carbonyl group in the candidate substance might be metabolised mainly via reduction

and conjugation, as described for the benzyl alkyl ketones. This anticipation is only partially supported by the results on studies carried out with substituted naphthalenes, such as 2-methyl-naphthalene (Teshima et al., 1983) and 2-isopropyl-naphthalene (Honda et al., 1987). Administration of 2-methyl-naphthalene to guinea pigs resulted in the urinary excretion of oxidative products of the methyl group (naphthoic acid and its glycine and glucuronic acid conjugates) although other metabolites (about 18 % of the totally excreted radioactivity) were also present, including glucuronic acid and sulphate conjugates of 7-methyl-1-naphtol, indicating the occurrence of ring hydroxylation (Teshima et al., 1983). Similarly, the administration of 2-isopropyl-naphthalene to rabbits resulted in urinary metabolites originating from oxidation of both the side-chain and of the naphthalene ring (Honda et al., 1987).

III.4. Summary and Conclusions

Based on the studies discussed above for the structurally related supporting substances, it may be concluded that the candidate substances 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], 3-hydroxy-4-phenylbutane-2-one [FL-no: 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are readily absorbed from the gut. Based on toxicokinetic data for the representative structurally related substance 4-phenyl-3-buten-2-one [FL-no: 07.024], it appears that orally administered phenyl alkyl ketones undergo essentially complete first-pass metabolism prior to systemic distribution. They can be reduced to the corresponding secondary alcohols, then either conjugated with glucuronic acid or glycine and excreted primarily in the urine. These ketones may also undergo omega-oxidation in the side-chain to yield intermediary metabolites (e.g. hydroxy-acetophenone) that undergo further oxidation and cleavage to yield aromatic carboxylic acids (phenylacetic acid or benzoic acid, depending on the number of carbon atoms in the side-chain). These aromatic acids are excreted primarily as glycine conjugates.

No information is available on the toxicokinetics of either the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214] or its supporting substance methyl 2-naphthyl ketone [FL-no: 07.013]. Although it could be hypothesised that the carbonyl group could be reduced and/or oxidised and then conjugated as described for the benzyl alkyl ketones, the occurrence of other metabolic pathways cannot be ruled out.

In conclusion, it can be anticipated that the six candidate substances 1-phenylbutan-1-one [FL-no: 07.193], 4-phenylbutan-2-one [FL-no: 07.194], 1-phenylpropan-2-one [FL-no: 07.195], 3-hydroxy-4-phenylbutane-2-one [FL-no: 07.242], 2-methoxy-acetophenone [FL-no: 07.254] and 2-methyl-acetophenone [FL-no: 07.259] are metabolised to innocuous product. This cannot be anticipated for the candidate substance alpha-methyl naphthyl ketone [FL-no: 07.214].

ANNEX IV: TOXICITY

Oral acute toxicity data are available for two candidate substances of the present Flavouring Group Evaluation from chemical group 21, and for 13 supporting substances evaluated by the JECFA at the 57th meeting. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(1-Phenylethan-1-ol [FL-no: 02.064])	Rat	M	Gavage	400	(Smyth & Carpenter, 1944)	2
(1-Phenethyl acetate [FL-no: 09.178])	Rat	NR	Oral	5000	(Posner, 1971)	2
(1-Phenethyl propionate [FL-no: 09.144])	Rat	M, F	Gavage	5.2 ml/kg bw	(Levenstein, 1973e)	2
(1-(p-Tolyl)ethan-1-ol [FL-no: 02.080])	Mouse	NR[1]	Oral[1]	2.8 ml/kg bw	(Linét et al., 1962.)	2
(Acetophenone [FL-no: 07.004])	Rat	M	Gavage	3000	(Smyth & Carpenter, 1944)	2
	Rat	M, F	Gavage	3200	(Jenner et al., 1964)	2
	Rat	F	Oral	2.48 ml/kg bw	(Smyth et al., 1969b)	2
	Rat	NR	Oral	900	(Smyth & Carpenter, 1948)	2
	Mouse	NR	Oral	1780	(Damment, 1992)	2
(4-Methylacetophenone [FL-no: 07.022])	Rat	M, F	Gavage	1400	(Industrial Bio-Test Laboratories, Inc., 1971b)	2
(4-Methoxyacetophenone [FL-no: 07.038])	Rat	NR	Oral	1720	(Moreno, 1973t)	2
(4-Methoxyphenylacetone [FL-no: 07.087])	Rat	NR	Oral	3.33 ml/kg bw	(Levenstein, 1976f)	2
4-Phenylbutan-2-one [FL-no: 07.194]	Rat	M	Oral	3200	(Moreno, 1980j)	
(4-(4-Methoxyphenyl)butan-2-one [FL-no: 07.029])	Rat	NR	Oral	>5000	(Russell, 1973f)	2
(1-Phenylpropan-1-ol [FL-no: 02.033])	Rat	NR	Gavage	2800	(Brown et al., 1955)	2
	Rat	NR	Gavage	2500	(Rohrbach & Robineau, 1958)	2
	Mouse	NR	Gavage	500	(Rohrbach & Robineau, 1958)	2
(1-Phenylpropan-1-one [FL-no: 07.040])	Rat	NR	Oral	4.49 ml/kg bw	(Carpenter et al., 1974)	2
(3-Benzylheptan-4-one [FL-no: 07.070])	Rat	M, F	Gavage	4400	(Burdock & Ford, 1990d)	2
	Rat	M, F	Gavage	4441	(Reagan & Becci, 1984c)	2
alpha-Methyl naphthyl ketone [FL-no: 07.214]	Rat	NR	Oral	1560	(Moreno, 1977x)	
	Rat	M	Oral	800	(Eastman Kodak Co., 1992b)	
	Mouse	M	Oral	800	(Eastman Kodak Co., 1992b)	
(Methyl 2-naphthyl ketone [FL-no: 07.013])	Mouse	NR	Oral	3100	(Moreno, 1982k)	2

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

[1]*Data point not verified.*

[2] *Summarised by JECFA 57th meeting (JECFA, 2002a).*

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical group 21 and for nine supporting substances evaluated by the JECFA at the 57th meeting. The supporting substances are listed in brackets.

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

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(1-Phenylethan-1-ol [FL-no: 02.064])	Mouse; M, F 9-10	Gavage	0, 125, 250, 500, 1000, 2000 mg/kg; 5day/week	16 days	500	(NTP, 1990d)	3
	Mouse; M, F 20	Gavage	0, 46.9, 93.8, 187.5, 375, 750 mg/kg	13 weeks	750[1]	(NTP, 1990d)	3
	Rat; M, F 10	Gavage	0, 125, 250, 500, 1000, 2000 mg/kg; 5day/week	16 days	1000	(NTP, 1990d)	3
	Rat; M, F 20	Gavage	0, 93, 187, 375, 750, 1500 mg/kg	13 weeks	187	(NTP, 1990d)	3
	Mouse;M, F 100	Gavage	0, 375, 750 mg/kg; 5day/week	103 weeks	375	(NTP, 1990d)	3
	Rat; M, F 100	Gavage	0, 375, 750 mg/kg; 5day/week	103 weeks	<375[2]	(NTP, 1990d)	3
(1-Phenethyl acetate [FL-no: 09.178])	Rat; M, F 30	Gavage	0, 15, 50, 150 mg/kg; 7day/week	13 weeks	15	(Gaunt et al., 1974)	3
(Acetophenone [FL-no: 07.004])	Rat; M, F 10	Diet	0, 100, 250, 1000 mg/kg;	17 weeks	1000[1]	(Hagan et al., 1967)	3
(1-Methyl-2-phenethyl butyrate [FL-no: 09.249])	Rat; M, F 20 - 32	Diet	Single dosage level	90 days	M: 3.09[1] F: 3.46[1]	(Posternak et al., 1969)	3
(4-(4-Methoxyphenyl)butan-2-one [FL.no: 07.029])	Rat; M 6	Diet	0, 0.5, 1, 2 % in diet	2 weeks	500	(Trubek Laboratories, Inc., 1956)	3
	Rat; M, F 20	Diet	56, 114 mg/kg/day	90 days	114[1]	(Trubek Laboratories, Inc., 1958e)	**3**
(1-Phenylpropan-1-ol [FL-no: 02.033])	Rat; M, F 10	Diet	Single dosage level	4 months	M: 415[1] F: 476[1]	(Brown et al., 1955)	3
(4-Methyl-1-phenylpentan-2-ol [FL-no: 02.065])	Rat; M, F 30	Oral	0, 10, 40, 160 mg/kg/day	13 weeks	10	(Ford et al., 1983)	3
(1-Phenylpropan-1,2-dione [FL-no: 07.079])	Rat; M, F 16	Diet	Single dosage level	90 days	M: 17.53[1] F: 17.26[1]	(Posternak et al., 1969)	3
alpha-Methyl naphthyl ketone [FL-no: 07.214]	Rat; M 5	Gavage	0, 100, 500, 1000 mg/kg	1000 mg/kg: 2 doses (2 days) 100 mg/kg: 13 doses (17 days) 500 mg/kg: 12 doses (16 days)	<100	(Eastman Kodak Co., 1992b)	No original data but interpretation such as “normal” or “increase/ decrease combined with slight/moderate/great” are given. Interpretation not comprehensible.
(Methyl 2-naphthyl ketone [FL-no: 07.013])	Rat; M, F 30	Diet	Single dosage level.	90 days	M: 33.0[1] F: 36.9[1]	(Oser et al., 1965)	**3**

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

2 Excessive reduction in body weights and prevalence of gavage-related deaths are significant inadequacies in the study.

3 Summarised by the JECFA 57th meeting (JECFA, 2002a).

Developmental and reproductive toxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical group 21 but for none of the supporting substances.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Species/ Sex No./ group	Route	Dose Levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
alpha-Methyl naphthyl ketone [FL-no: 07.214]	Rat; NR 5 (1st generation), 8 (2nd generation)	Diet	Single dose level	Two-generation Reproductive Toxicity: 8 months, 2 mg every second day	2 mg[1, 2]	(Sporn et al., 1963)	Insufficient quality. Details of methods and on purity of test material not reported.

NR: Not reported

[1] *This study was conducted at a single dose level that produced no adverse effects.*

[2] *Test material identified as methyl naphthyl ketone (probably a commercial mixture of methyl alpha-naphthyl ketone and methyl beta-naphthyl ketone). No effects were noted in a two-generation reproduction screening study in rats. First generation pregnant animals were dosed every other day with 2 mg/kg/bw of methyl naphthyl ketone.*

In vitro mutagenicity/genotoxicity data are available for two candidate substances of the present Flavouring Group Evaluation from chemical group 21 and for nine supporting substances evaluated by the JECFA at the 57th meeting. Supporting substances are listed in brackets.

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

Chemical Name [FL-no]	Test System	Test Object	Maximum Concentration	Result	Reference	Comments
(1-Phenylethan-1-ol [FL-no: 02.064])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	6666 µg/plate	Negative[1, 2]	(NTP, 1990d)	4
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	6666 µg/plate	Negative[1, 2]	(Zeiger et al., 1987)	4
	Forward mutation assay	Mouse lymphoma L5178Y/TK+/-	750 nl/ml	Positive[2]	(NTP, 1990d)	
	Sister chromatid exchange	Chinese hamster ovary cells	1000 µg/ml	Negative[1, 2]	(NTP, 1990d)	4
	Chromosomal aberration	Chinese hamster ovary cells	2500 µg/ml 4000 µg/ml	Negative[1] Positive[2]	(NTP, 1990d)	4
(Acetophenone [FL-no: 07.004])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	360 µg/plate	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test, only one concentration tested).
	Ames test	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1, 2]	(Fujita & Sasaki, 1987)	4
	Ames test	*S. typhimurium* TA98; TA100; TA2637	1000 µg/plate	Negative[1, 2]	(Nohmi et al., 1985)	4
	Chromosomal aberration	Chinese hamster ovary cells	1200 µg /ml 1000 µg /ml	Negative[1] Positive[2]	(Sofuni et al., 1985)	4
1-Phenylbutan-1-one [FL-no: 07.193]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate[3]	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test, only one concentration tested).
(4-Methoxyacetophenone [FL-no: 07.038])	Modified Ames test	*E. coli* WP2; WP2*uvr*A-	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	4
	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538; D3052; C3076; G45	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	4
4-Phenylbutan-2-one [FL-no: 07.194]	Ames test	*S. typhimurium* TA100	100 µg/plate	Negative[1, 2]	(Kitamura et al., 1999)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (only one bacterial strain tested, test substance concentration not reported, result not reported in detail).
(4-(4-Methoxyphenyl)butan-2-one [FL-no: 07.029])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1, 2]	(Wild et al., 1983)	Details of methods and results were not reported. Validity cannot be evaluated.
(1-phenylpropan-1-one [FL-no:	Modified Ames test	*E. coli*	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	4

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

Chemical Name [FL-no]	Test System	Test Object	Maximum Concentration	Result	Reference	Comments
07.040])		WP2; WP2*uvr*A-				
	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538; G46; C3076; D3052	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	4
(1-Phenylpentan-2-ol [FL-no: 02.034])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1, 2]	(Wild et al., 1983)	Details of methods and results were not reported. Validity cannot be evaluated.
(Ethyl 3-phenyl-3-oxopropionate [FL-no: 09.476])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1, 2]	(Wild et al., 1983)	Details of methods and results were not reported. Validity cannot be evaluated.
(1-Phenylpropan-1,2-dione [FL-no: 07.079])	Ames test	*S. typhimurium* TA100	148 µg/plate	Negative[1]	(Dorado et al., 1992)	4
(Methyl 2-naphthyl ketone [FL-no: 07.013])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1, 2]	(Wild et al., 1983)	Details of methods and results were not reported. Validity cannot be evaluated.
	Ames test	*S. typhimurium* TA97; TA102	100µg/plate	Negative[1, 2]	(Fujita et al., 1992)	4

[1] *Without S9 metabolic activation.*

[2] *With S9 metabolic activation.*

[3] *Spot test protocol. In a confirmatory assay, the test substance was quantitatively evaluated in TA100 at up to 3 µmol/plate.*

[4] *Summarised by JECFA 57th meeting (JECFA, 2002a).*

In vivo mutagenicity/genotoxicity data are available for none of the candidate substance of the present Flavouring Group Evaluation from chemical group 21 but for two supporting substances evaluated by the JECFA at the 57th meeting.

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

Chemical Name [FL-no]	Test system	Test Object	Dose	Result	Reference	Comments
(4-(4-Methoxyphenyl)butan-2-one [FL-no: 07.029])	*In vivo* Micronucleus test	Mouse bone marrow cells	1426 mg/kg bw by i.p.	Negative	(Wild et al., 1983)	Study design does not meet the criteria of current guidelines (bone marrow was sampled only once after dosing, PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow). Not in accordance with OECD guideline 474 (1983/1997).
(Methyl 2-naphthyl ketone [FL-no: 07.013])	*In vivo* Micronucleus test	Mouse bone marrow cells	876 mg/kg bw by i.p.	Negative	(Wild et al., 1983)	Study design does not meet the criteria of current guidelines (bone marrow was sampled only once after dosing, PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow). Not in accordance with OECD guideline 474 (1983/1997).

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
GSH	Glutathione
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavour Industry
IP	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
LOAEL	Lowest Observed Adverse Effect Level
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
MTD	Maximum Tolerated Dose
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
NMR	Nuclear Magnetic Resonance
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-aperation and Development

SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
WHO	World Health Organisation