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SCIENTIFIC OPINION

Flavouring Group Evaluation 7, Revision 2 (FGE.07Rev2)[1]: Saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids from chemical group 5

Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2009-00478)

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PANEL MEMBERS

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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 43 flavouring substances in the Flavouring Group Evaluation 7, Revision 2 (FGE.07Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 43 flavouring substances belong to chemical group 5, Annex I of the Commission Regulation (EC) No 1565/2000.

[1] For citation purposes: Scientific Opinion of the Panel on Food Conact Materials, Enzymes, Flavourings and Processing Aids (CEF) on a request from the Commission on Flavouring Group Evaluation 7 revision 2. *The EFSA Journal* (2009) 1020, 1-70.

The present Flavouring Group Evaluation (FGE) deals with 43 saturated and unsaturated aliphatic secondary alcohols, ketones and esters with a secondary alcohol moiety.

Twenty-two of the 43 candidate substances possess a chiral centre and two of the candidate substances possess two chiral centres. For six of these substances the stereoisomeric composition has not been specified.

Due to the presence and the position of double bonds, six of the substances can exist as geometrical isomers. In three of these cases the stereoisomeric composition has not been specified.

Twenty-eight of the flavouring substances have been assigned to structural class I and 15 substances belong to structural class II.

Thirty-nine of the flavouring substances in the present group of 43 flavouring substances have been reported to occur naturally in a wide range of food items.

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

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

According to the default MSDI approach, the 43 flavouring substances in this group have intakes in Europe from 0.0012 to 1.3 microgram/capita/day, which are below the threshold of concern value for both structural class I (1800 microgram/person/day) and structural class II (540 microgram/person/day) substances.

On the basis of available data from *in vitro* and *in vivo* tests on candidate and supporting substances, it can be concluded that the 43 flavouring substances included in this group exhibit no genotoxic potential.

Among the 43 flavouring substances, 42 would be expected to be metabolised to innocuous products at the estimated levels of intake as flavouring substances. The remaining flavouring substance, 5-methylheptan-3-one [FL-no: 07.182], may be oxidised to a potential neurotoxic gamma-diketone. However, this metabolic path does not pose a safety concern at the estimated level of intake as a flavouring substance. Indeed, for this substance a No Observed Adverse Effect Level (NOAEL) for neurotoxicity was established, which provided a large margin of safety in relation to the estimated level of intake of the flavouring substance.

Otherwise it was noted, that where toxicity data were available on single flavouring substances, they were consistent with the conclusions in the present Flavouring Group Evaluation (FGE) using the Procedure.

Therefore, it was considered that on the basis of the default MSDI approach none of the 43 flavouring substances would give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3900 microgram/person/day for the 28 candidate substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for three flavouring substances [FL-no: 07.084, 07.178 and 07.239]. The estimated intakes of the 15 candidate substances assigned to structural class II, based on the mTAMDI, range from 1500 to 1600 microgram/person/day, which are all above the threshold of concern for structural class II of 540 microgram/person/day. The three substances [FL-no: 07.084, 07.178 and 07.239], which have mTAMDI intake estimates below the threshold of concern for the structural class, are also expected to be metabolised to innocuous products.

Thus, for 40 of the 43 candidate substances considered in this Opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these 40 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

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

Adequate specifications including purity and identity for the materials of commerce have been provided for 35 of the 43 flavouring substances. Information on stereoisomerism has not been provided for eight of the 43 substances [FL-no: 02.182, 02.190, 02.255, 07.156, 07.236, 09.676, 09.880 and 09.926].

Thus, the final evaluation of the materials of commerce cannot be performed for these eight substances, pending further information. The remaining 35 substances [FL-no: 02.077, 02.124, 02.142, 02.148, 02.177, 02.183, 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.162, 07.178, 07.181, 07.182, 07.185, 07.189, 07.199, 07.201, 07.205, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608 and 09.609] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

KEYWORDS

Flavourings, safety, saturated, unsaturated, secondary alcohols, ketones, carboxylic acids, esters.

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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 2009/163/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 Flavouring Group Evaluation (FGE) is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the first FGE.

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

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

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No of candidate substances
FGE.07	9 December 2004	http://www.efsa.eu.int/science/afc/afc_opinions/813_en.html	35
FGE.07Rev1	26 September 2007	http://www.efsa.europa.eu/EFSA/ScientificPanels/AFC/efsa_locale-1178620753812_Opinions425.htm	41
FGE.07Rev2	26 March 2009	http://www.efsa.europa.eu/EFSA/ScientificOpinionPublicationReport/efsa_locale-1178620753812_ScientificOpinions.htm	43

The present revision of FGE.07, FGE.07Rev2, includes the assessment of two additional flavouring substances [FL-no: 02.255 and 07.239]. No new data on toxicity and metabolism have been provided.

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a positive list

according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

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ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 7, Revision 2

1.1. Description

The present Flavouring Group Evaluation 7, Revision 2 (FGE.07Rev2), using the Procedure as referred to in the Commission Regulation (EC) 1565/2000 (the Procedure - shown in schematic form in Annex I), deals with 43 saturated and unsaturated aliphatic acyclic secondary alcohols, ketones and esters with a secondary alcohol moiety. These 43 flavouring substances belong to the chemical group 5 of Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 43 flavouring substances (candidate substances) are closely related structurally to 52 flavouring substances (supporting substances) evaluated at the 51st and 59th meeting of the Joint FAO/WHO Expert Committee on Food Additives (the JEFCA) in the group "Saturated Aliphatic Acyclic Secondary Alcohols, Ketones, and Related Saturated and Unsaturated Esters" (JECFA, 2000a; JECFA, 2002c).

The 43 candidate substances under consideration in the present evaluation are listed in Table 1, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, and structures. Seven out of the 43 flavouring substances are saturated aliphatic acyclic secondary alcohols [FL-no: 02.077, 02.142, 02.148, 02.177, 02.182, 02.183 and 02.190]; two are unsaturated aliphatic secondary alcohols [FL-no: 02.124 and 02.255]; 13 are saturated aliphatic ketones [FL-no: 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.178, 07.181, 07.182, 07.185, 07.189, 07.199 and 07.205]; five are unsaturated aliphatic ketones [FL-no: 07.156, 07.162, 07.201, 07.236 and 07.239] of which three contain a terminal double bond [FL-no: 07.162, 07.201 and 07.239] and 16 are esters of aliphatic acyclic secondary alcohols and linear or branched chain aliphatic carboxylic acids [FL-no: 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926].

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

The names and structures of the 52 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; SCF, 1995; JECFA, 1999a; JECFA, 2002c).

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.).

Twenty-two of the 43 candidate substances possess a chiral centre [FL-no: 02.124, 02.142, 02.148, 02.177, 02.183, 02.190, 02.255, 07.157, 07.182, 07.185, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.676, 09.880 and 09.926] and two of the candidate substances possess two chiral centres [FL-no: 02.182 and 07.205]. For six of these substances [FL-no: 02.182, 02.190, 02.255, 09.676, 09.880 and 09.926], the stereoisomeric composition has not been specified (see Table 1).

Due to the presence and the position of double bonds, six of the 43 candidate substances can exist as geometrical isomers [FL-no: 02.255, 07.156, 07.236, 07.239, 09.386 and 09.880]. In three of these cases [FL-no: 07.156, 07.236 and 09.880], the stereoisomeric composition has not been specified (see Table 1).

For two of the flavouring substances [FL-no: 02.182 and 09.880] Industry has informed that they exist as a "mixture of isomers". However, the Panel does not consider this information sufficient and requests data on the actual ratios.

1.3. Natural Occurrence in Food

Thirty-nine of the candidate substances in the present group of 43 candidate substances have been reported to occur naturally. The natural products in which these candidate substances are reported to occur mainly are: meat products (chicken, guinea hen), milk products (butter, milk powder, cheese), fruits (apricot, banana, pineapple, guava, mango, grapefruit, cocoa, strawberry, papaya, passion fruit, mushroom, tomato, sweet corn, green tea), alcoholic beverages (grape brandy, beer, white wine), and/or herbs and spices (dill, lemon balm, clove bud) (TNO, 2000). For the food products mentioned above, quantitative data have been reported for 20 substances in the present Flavouring Group Evaluation. These reports include among others:

- 3-methylpentan-2-ol [FL-no: 02.182]: 0.009 mg/kg in pineapple
- heptadecan-2-one [FL-no: 07.160]: 0.1 mg/kg in blue cheese, 1.1 mg/kg in cocoa, and 8.7 mg/kg in heated butter
- pentan-3-one [FL-no: 07.084]: up to 14 mg/kg in different mushroom
- sec-butyl acetate [FL-no: 09.323]: up to 67 mg/kg in vinegar
- 1-methylhexyl acetate [FL-no: 09.388]: 400 mg/kg in clove bud
- 6,10,14-trimethylpentadecan-2-one [FL-no: 07.205]: 2000 mg/kg in lemon balm.

According to TNO four substances, [R-(E)]-5-isopropyl-8-methylnona-6,8-dien-2-one [FL-no: 07.239], octan-3-yl formate [FL-no: 09.926], sec-butyl hexanoate [FL-no: 09.332] and 4-hepten-2-yl butyrate [FL-no: 09.880], have not been reported to occur naturally in any food items (TNO, 2000).

2. Specifications

Purity criteria for the 43 candidate substances have been provided by the Flavour Industry (EFFA, 2001a; EFFA, 2002b; EFFA, 2002f; EFFA, 2007k; Flavour Industry, 2006p).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the information is adequate for 35 of the 43 candidate substances. Information on geometrical stereoisomerism and/or chirality is needed for eight candidate substances (see Section 1.2 and Table 1).

3. Intake Data

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

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

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

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

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

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified TAMDI approach (mTAMDI) 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 Maximised Survey-derived Daily Intake (MSDI (SCF, 1999)) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[2] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation 7, Revision 2 (FGE.07Rev2) the total annual volume of production of the 43 candidate substances for use as flavouring substances in Europe has been reported to be approximately 80 kg (EFFA, 2002e; EFFA, 2002f; EFFA, 2007k) and for 39 of the 52 supporting substances approximately 750000 kg (isopropyl alcohol accounts for 690000 kg and acetone for 50000 kg) (cited by the JECFA (JECFA, 1999a)) and around 1300 kg for 10 of the remaining 13 of the 52 supporting substances (JECFA, 2003a).

On the basis of the annual volumes of production reported for of the 43 candidate substances, the daily per capita intakes for each of these flavourings have been estimated (Table 2a). Approximately 65 % of the total annual volume of production for the candidate substances (EFFA, 2002e; EFFA, 2002f; EFFA, 2007k) is accounted for by the following seven flavourings: isopropyl octanoate [FL-no: 09.608], sec-butyl butyrate [FL-no: 09.325], 3-methylpentan-2-one [FL-no: 07.185], hept-4-enyl-2-butyrate [FL-no: 09.880], dodecan-2-one [FL-no: 07.158], nonan-4-one [FL-no: 07.189] and decan-2-one [FL-no: 07.150]. The estimated daily per capita intakes of these candidate substances from use as a flavouring substance are 1.3, 1.3, 1.2, 0.8, 0.7, 0.5, and 0.5 microgram, respectively. The daily per capita intakes for the remaining substances is 3.4 microgram (Table 2a).

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

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

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the 43 candidate substances, information on food categories and normal and maximum use levels[3,4,5] were submitted by the Flavour Industry (EFFA, 2002b; EFFA, 2002f; EFFA, 2007a; EFFA, 2007b; EFFA, 2007k; Flavour Industry, 2006p). The 43 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex

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

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

[4] *The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).*

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

III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as summarised in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 01.0	Dairy products, excluding products of category 2	All 43
Category 02.0	Fats and oils, and fat emulsions (type water-in-oil)	All 43
Category 03.0	Edible ices, including sherbet and sorbet	All 43
Category 04.1	Processed fruits	All 43
Category 04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 05.0	Confectionery	All except [FL-no: 07.205]
Category 06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 02.255]
Category 07.0	Bakery wares	All 43
Category 08.0	Meat and meat products, including poultry and game	All except [FL-no: 02.255]
Category 09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 09.608 & 02.255]
Category 10.0	Eggs and egg products	None
Category 11.0	Sweeteners, including honey	None
Category 12.0	Salts, spices, soups, sauces, salads, protein products, etc.	All except [FL-no: 07.156 & 02.255]
Category 13.0	Foodstuffs intended for particular nutritional uses.	All 43
Category 14.1	Non-alcoholic ("soft") beverages, excluding dairy products	All 43
Category 14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	All except [FL-no: 07.205]
Category 15.0	Ready-to-eat savouries	All except [FL-no: 02.255, 07.157 & 09.609]
Category 16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 43

According to the Flavour Industry the normal use levels for the 43 candidate substances are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 to 100 mg/kg (EFFA, 2002b; EFFA, 2002f; EFFA, 2002i;EFFA, 2007a; EFFA, 2007b; EFFA, 2007k; Flavour Industry, 2006p).

The mTAMDI values for the 28 candidate substances from structural class I (see Section 5) range from 1600 to 3900 microgram/person/day. For the 15 candidate substance from structural class II the mTAMDI range from 1500 to 1600 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

In general, aliphatic secondary alcohols and ketones are expected to be rapidly absorbed in the gastrointestinal tract. The candidate aliphatic esters are expected to be hydrolysed enzymatically to their component secondary alcohols and carboxylic acids. The carboxylic acids are completely oxidised in the fatty acid pathway and the tricarboxylic acid pathway (see Annex III).

Secondary alcohols may undergo oxidation to the corresponding ketone; however, in the *in vivo* situation the alcohol is removed from the equilibrium by conjugation to glucuronic acid, which represents the major pathway of metabolism for secondary alcohols. The glucuronides of the candidate secondary alcohols are expected to be eliminated via the urine (Felsted & Bachur, 1980; Kasper & Henton, 1980; JECFA, 1999a).

In general, the major metabolic pathway for aliphatic ketones is reduction of the ketone to the corresponding secondary alcohol and subsequent excretion as glucuronic acid conjugate (Felsted & Bachur, 1980; JECFA, 1999a).

Short chain ketones (C < 5) that contain a carbonyl function at the C2 position may undergo oxidation to yield an alpha-ketocarboxylic acid, which through decarboxylation will be oxidised to carbon dioxide and a simple aliphatic carboxylic acid that will enter the fatty acid pathway and citric acid cycle (Dietz et al., 1981). Ketones may also be metabolised by omega- or omega-1-oxidation yielding a hydroxyketone that may be further reduced to a diol and excreted in the urine as glucuronic acid conjugate. Longer chain aliphatic ketones (C ≥ 5) are primarily metabolised via reduction, but omega- and omega-1-oxidation are competing pathways at high concentrations (Dietz et al., 1981; Topping et al., 1994).

Omega-1-oxidation of certain aliphatic ketones may yield gamma-diketones, which may give rise to neuropathy of giant axonal type. The metabolic pathway includes oxidation of the omega-1-carbon, first to a hydroxyketone and then to a diketone. The gamma-spacing of the carbonyl functions has been shown to be a prerequisite for neurotoxic effects, thus, only ketones with this structural feature may yield the neurotoxic metabolites. Neurotoxic effects are however only observed at relatively high dosages (Topping et al., 1994). One of the candidate substances, 5-methylheptan-3-one [FL-no: 07.182] may potentially be oxidised to a gamma-diketone.

Three of the candidate substances, hex-5-en-2-one, tridec-12-en-2-one and ([R-(E)]-5-isopropyl-8-methylnona-6,8-dien-2-one [FL-no: 07.162, 07.201 and 07.239], have terminal double bonds. These double bonds may be oxidised to the corresponding epoxides. Epoxides are highly reactive molecules, due to the large strain associated with the three membered ring structure, and they react easily with nucleophilic sites of cellular macromolecules. For this reason, several aliphatic alkene-derived epoxides (e.g. ethylene, isoprene, butadiene, and glycidol) have been demonstrated to be carcinogenic (Melnick, 2002). However, epoxides can be conjugated with glutathione by glutathione S-transferases or hydrolysed to diols by epoxide hydrolases. The latter two reactions can be considered to be detoxications. 1-Alkenes are metabolised by P450 through both double bond oxidation to the corresponding epoxide and allylic oxidation (Chiappe et al., 1998). The rates of the two reactions measured with different P450 isoforms indicate that epoxide formation is generally favoured (Chiappe et al., 1998). Therefore, due to the similar position of the double bond, it cannot be ruled out that, in addition to the above mentioned metabolic pathways for ketones, the three

candidate substances [FL-no: 07.162, 07.201 and 07.239] may be, at least partially, biotransformed to an epoxide.

However, based on the low levels of intake of alkenones characterised by a carbonyl group in a distant position to the terminal double bond, it is expected that the detoxication reactions would not be saturated and would outweigh the rate of epoxide formation.

The presence of the terminal double bond in these three candidate substances is therefore not considered of concern because epoxides can be detoxicated by conjugation with glutathione or by epoxide hydrolase mediated hydrolysis.

Furthermore, based on genotoxicity data available for seven out of 48 flavouring substances with terminal double bonds from the Register (EC, 1999a; EC, 2004a) it is not indicated that a terminal double bond distal to a functional group is a structural alert for genotoxicity.

In addition to reduction and oxidation pathways, low molecular weight ketones may be excreted unchanged in expired air (Brown et al., 1987).

Concluding Remarks on Metabolism

Among the candidate substances seven saturated aliphatic acyclic secondary alcohols, two unsaturated aliphatic secondary alcohol, 13 saturated aliphatic ketones, five unsaturated aliphatic ketones and 16 esters of aliphatic acyclic secondary alcohols and linear and branched chain aliphatic carboxylic acids may be expected to be metabolised to innocuous substances at the estimated level of intake, based on the MSDI approach, as flavouring substances.

Three of the candidate substances [FL-no: 07.162, 07.201 and 07.239] contain terminal double bonds. However, the presence of terminal double bonds in these three substances is not considered of concern, because these double bonds may be oxidised to the corresponding epoxides, which can be detoxicated by conjugation with glutathione or by epoxide hydrolase mediated hydrolysis.

One candidate substance, 5-methylheptan-3-one [FL-no: 07.182], may be oxidised to a potentially neurotoxic gamma-diketone.

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 43 candidate substances the Procedure as outlined in Annex I was applied. The stepwise evaluations of the 43 substances are summarised in Table 2a.

Step 1.

Twenty-eight of the candidate substances [FL-no: 02.077, 02.124, 02.142, 02.148, 02.177, 02.182, 02.183, 02.190, 02.255**,** 07.084, 07.178, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386,

09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926] are classified in structural class I, according to the decision tree approach presented by Cramer et al. (1978). The remaining 15 candidate substances [FL-no: 07.072, 07.150, 07.156, 07.157, 07.158, 07.160, 07.162, 07.181, 07.182, 07.185, 07.189, 07.199, 07.201, 07.205 and 07.236], which are acyclic aliphatic saturated or unsaturated ketones, are in structural class II.

Step 2.

Forty-two of the 43 candidate substances were considered to be metabolised to innocuous products and would not be expected to saturate available detoxification pathways at estimated levels of intake, based on the MSDI approach, from use as flavouring substances. Therefore, these 42 substances proceed via the A-side of the Procedure scheme (Annex I).

One candidate substance, 5-methylheptan-3-one [FL-no: 07.182], cannot be predicted to be metabolised to innocuous products and therefore, proceeds to step B3.

Step A3.

The 28 candidate substances assigned to structural class I, have estimated European daily per capita intakes ranging from 0.0012 to 1.3 microgram (Table 2a). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

The 15 ketones, which have been assigned to structural class II, have estimated European daily per capita intakes ranging from 0.0012 to 1.2 microgram (Table 2a). These intakes are below the threshold of concern of 540 microgram/person/day for structural class II.

Based on results of the safety evaluation sequence the 42 candidate substances proceeding via the A-side of the Procedure do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

Step B3.

The estimated per capita intake of 5-methylheptan-3-one [FL-no: 07.182] (0.32 microgram/ capita/day) does not exceed the threshold of concern for structural class II (540 microgram/ person/day). Accordingly, the candidate substance proceeds to step B4 of the Procedure.

Step B4.

On the basis of a study on the neurotoxic effects of orally administered 5-methylheptan-3-one [FL-no: 07.182] to male rats, a NOAEL of 82 mg/kg body weight (bw)/day was established (IBM Corp., 1989). This NOAEL provides a large margin of safety in relation to level of estimated intake of the candidate substance (0.32 microgram/capita/day).

Based on results of the safety evaluation sequence this candidate substance does not pose a safety concern when used as flavouring substance at the estimated level of intake, based on the MSDI approach.

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

The estimated intakes for the 28 candidate substances in structural class I based on the mTAMDI approach range from 1600 to 3900 microgram/person/day. For three of these 28 substances the mTAMDI is below the threshold of concern of 1800 microgram/person/day. For comparison of the intake estimate based on the MSDI approach and mTAMDI approach, see Table 6.1.

The estimated intake for the 15 candidate substances assigned to structural class II based on the mTAMDI range from 1500 to 1600 microgram/person/day, which are all above the threshold of concern for structural class II substances of 540 microgram/person/day. For comparison of the MSDI and mTAMDI values, see Table 6.1.

For 40 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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

FL-no	EU Register name	MSDI (µg/capita/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.077	Pentan-3-ol	0.19	3900	Class I	1800
02.124	6-Methylhept-5-en-2-ol	0.0061	3900	Class I	1800
02.142	3,3-Dimethylbutan-2-ol	0.24	3900	Class I	1800
02.148	Dodecan-2-ol	0.35	3900	Class I	1800
02.177	2-Methylhexan-3-ol	0.12	3900	Class I	1800
02.182	3-Methylpentan-2-ol	0.12	3900	Class I	1800
02.183	4-Methylpentan-2-ol	0.0012	3900	Class I	1800
02.190	Nonan-3-ol	0.011	3900	Class I	1800
02.255	(Z)-4-Hepten-2-ol	0.03	2500	Class I	1800
07.084	Pentan-3-one	0.24	1600	Class I	1800
07.178	3-Methylbutan-2-one	0.073	1600	Class I	1800
07.239	[R-(E)]-5-Isopropyl-8-methylnona-6,8-dien-2-one	0.24	1600	Class I	1800
09.304	sec-Heptyl isovalerate	0.0012	3900	Class I	1800
09.323	sec-Butyl acetate	0.0012	3900	Class I	1800
09.325	sec-Butyl butyrate	1.3	3900	Class I	1800
09.328	sec-Butyl formate	0.12	3900	Class I	1800
09.332	sec-Butyl hexanoate	0.024	3900	Class I	1800
09.386	sec-Hept-4(cis)-enyl acetate	0.024	3900	Class I	1800
09.388	sec-Heptyl acetate	0.12	3900	Class I	1800
09.391	sec-Heptyl hexanoate	0.12	3900	Class I	1800
09.604	Isopropyl decanoate	0.12	3900	Class I	1800
09.605	Isopropyl dodecanoate	0.12	3900	Class I	1800
09.606	Isopropyl hexadecanoate	0.012	3900	Class I	1800
09.608	Isopropyl octanoate	1.3	3900	Class I	1800
09.609	Isopropyl valerate	0.012	3500	Class I	1800
09.676	sec-Octyl acetate	0.011	3900	Class I	1800
09.880	Hept-4-enyl-2 butyrate	0.79	3900	Class I	1800
09.926	Octan-3-yl formate	0.24	3900	Class I	1800
07.072	6-Methylheptan-3-one	0.19	1600	Class II	540
07.150	Decan-2-one	0.52	1600	Class II	540
07.156	2,6-Dimethyloct-6-en-3-one	0.0012	1600	Class II	540
07.157	6,10-Dimethylundecan-2-one	0.085	1500	Class II	540
07.158	Dodecan-2-one	0.73	1600	Class II	540
07.160	Heptadecan-2-one	0.12	1600	Class II	540
07.162	Hex-5-en-2-one	0.049	1600	Class II	540
07.181	6-Methylheptan-2-one	0.0012	1600	Class II	540
07.185	3-Methylpentan-2-one	1.2	1600	Class II	540
07.189	Nonan-4-one	0.52	1600	Class II	540
07.199	Tetradecan-2-one	0.073	1600	Class II	540
07.201	Tridec-12-en-2-one	0.024	1600	Class II	540
07.205	6,10,14-Trimethylpentadecan-2-one	0.0073	1500	Class II	540
07.236	5-Octen-2-one	0.0097	1600	Class II	540
07.182	5-Methylheptan-3-one	0.32	1600	Class II	540

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

The 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, 2002e; EFFA, 2002f; EFFA, 2007k), the total estimated daily per capita intake as flavourings of the 28 candidate flavouring substances assigned to structural class I is 6 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. For the combined intake of the 15 candidate flavouring substances assigned to structural class II is 3.9 microgram, which does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

The 43 candidate substances are structurally related to 52 supporting substances evaluated by JEFCA at its 51st meeting (JECFA, 1999a) and 59th meeting (JECFA, 2003a). If the supporting substances of structural class I or II were consumed concomitantly on a daily basis, the total estimated combined intake (in Europe) would exceed the human thresholds of concern for structural classes I and II, respectively. However, the major contribution (>98 %) was provided by two supporting substances, namely acetone [FL-no: 07.050] (6.1 mg/capita/day) and isopropanol [FL-no: 02.079] (84 mg/capita/day). These are present in the body as endogenous compounds, which are easily eliminated from the body either by excretion into the urine and exhaled air or after enzymatic metabolism (Morgott, 1993). Therefore, they would not be expected to give rise to perturbations outside the physiological range (JECFA, 1999a). Excluding the two major contributors, the estimated total combined intake (in Europe) for the candidate and supporting substances belonging to structural class I would be 340 microgram/capita/day, which does not exceed the threshold of concern for the corresponding structural class (1800 microgram/ person/day); whereas the estimated total combined intake (in Europe) for the candidate and supporting substances belonging to structural class II would be 1080 microgram/capita/day, which is two fold higher than the threshold of concern for the corresponding structural class (540 microgram/person/day). However, these levels may be expected not to saturate the detoxification reactions involved in biotransformation of these compounds to innocuous products.

In the case that the candidate substance 5-methylheptan-3-one [FL-no: 07.182] and the two supporting substances heptan-3-ol [FL-no: 02.044] and 3-heptanone [FL-no: 07.003], which can all be metabolised to neurotoxic gamma-diketones, were consumed concomitantly on a daily basis the estimated combined intake (in Europe) would be 3.7 microgram/capita/day, corresponding to 0.06 microgram/kg bw/day. This value does not exceed the threshold of concern for the corresponding

structural class II (540 microgram/person/day) and is also much lower than the NOAEL for 5-methylheptan-3-one [FL-no: 07.182] of 82 mg/kg bw/day for neurotoxicity in the rat. Therefore, it can be concluded that there is no safety concern for human health for the combined exposure to these three neurotoxic substances at the estimated level of intake as flavourings.

8. Toxicity

8.1. Acute Toxicity Studies

Data are available for ten candidate substances under consideration and for 24 supporting substances. Most of the candidate and supporting substances have rat and/or mouse oral LD_{50} values exceeding 2000 mg/kg body weight (bw) indicating that their oral acute toxicity is low.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data on oral subchronic toxicity are available for two candidate substances pentan-3-one [FL-no: 07.084] and 5-methylheptan-3-one [FL-no: 07.182] with identification of a No Observed Adverse Effect Level (NOAEL); data on subacute and subchronic oral toxicity are also available for 10 supporting substances: one saturated aliphatic secondary alcohol [FL-no: 02.079], seven saturated [FL-no: 07.002, 07.003, 07.017, 07.020, 07.050, 07.058, 07.122] and two unsaturated [FL-no: 07.100 and 07.114] aliphatic ketones evaluated by JEFCA (JECFA, 1999a; JECFA, 2003a).

During the application of the Procedure (Annex I), the following study on methyl-5-heptan-3-one [FL-no: 07.182], which possesses structural alerts for neurotoxicity has been used to calculate the NOAEL.

5-Methyl-3-heptanone [FL-no: 07.182] (purity 98.9 %) dissolved in distilled water was administered by gavage to groups of five adult male Sprague Dawley rats at dose levels 0, 82, 410 and 820 mg/kg bw/day, five days/week for 13 weeks.

In the high-dose group clinical signs, including depression of activity, gait disturbances, reductions in food consumption and body weight gain were observed; moreover, results of the Functional Observational Battery (FOB) indicated peripheral neuropathy. Similar clinical signs and functional deficits were observed less frequently and with reduced severity in the mid-dose group. No functional deficits were observed in the low-dose group animals. Microscopic histopathological examinations of the sciatic and tibial nerves from high-dose animals revealed lesions typical of the "giant" axonal neuropathy produced by gamma-diketones. Changes observed in the mid-dose group animals reflected the occurrence of reparative processes in the nerves. Nerves from the low-dose group animals did not show any evidence of pathology attributable to treatment. Based on behavioural effects and microscopic changes occurring at 410 and 820 mg/kg bw/day, the NOAEL for methyl-5-heptan-3-one-induced neurotoxicity was 82 mg/kg bw/day (IBM Corp., 1989).

The repeated-dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental/Reproductive Toxicity Studies

Data on reproductive toxicity are available for one candidate substance, pentan-3-one [FL-no: 07.084]. For one supporting substance isopropyl alcohol [FL-no: 02.079] data are available on both developmental and reproductive toxicity.

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

8.4. Genotoxicity Studies

In vitro genotoxicity data have been reported for seven of the candidate substances. Negative results were obtained in bacterial systems (+/- metabolic activation) with four candidate substances: one saturated aliphatic acyclic secondary alcohol [FL-no: 02.183]; two saturated ketones [FL-no: 07.181 and 07.205]; and the ester isopropyl hexadecanoate [FL-no: 09.606]. Negative results were also obtained with two additional candidate substances, pentan-3-ol [FL-no: 02.077] and methyl-3-butan-2-one [FL-no: 07.178], tested for chromosomal aberrations in mammalian cells and induction of aneuploidy in yeast cells, respectively.

Induction of aneuploidy in yeast cells has been demonstrated for pentan-3-one [FL-no: 07.084]. The effect, measured only at high concentrations, approaching cytotoxic levels, can be considered to be a threshold effect, not mediated by direct interaction with DNA. In addition, induction of aneuploidy described in the paper is strongly potentiated by ice treatments included in the experimental protocol, consistently with tubulin dissociation at low temperature *in vitro*; in the absence of this passage the effect is very weak. Therefore, the effect could be considered as an effect occurring only under unrealistic experimental conditions and the extrapolation of this result to the *in vivo* situation in humans is questionable. Furthermore, it is well recognised that the relevance of fungal systems is limited when induction of aneuploidy in mammalian systems has to be evaluated.

In vitro genotoxicity data are also available for nine supporting substances.

No evidence of mutagenicity obtained with either bacterial or mammalian cells systems was reported for one saturated aliphatic acyclic secondary alcohol [FL-no: 02.079], four saturated [FL-no: 07.050, 07.017, 07.053 and 07.122] and one unsaturated [FL-no: 07.015] aliphatic acyclic ketones; and two esters of an aliphatic acyclic secondary alcohol with linear aliphatic carboxylic acids [FL-no: 09.003 and 09.105]. 4-Methyl-2-pentanone [FL-no: 07.017] gave negative results also when tested for chromosomal aberration activity.

Beside the negative results in *in vitro* bacterial point mutation tests, acetone [FL-no: 07.050] showed no evidence of increased sister chromatid exchanges in several cytogenetic assays on different mammalian cells, as well as no induction of chromosomal aberrations in Chinese hamster ovary cells up to very high concentrations. Only one test on hamster lung fibroblasts (conducted at an unspecified acetone concentration) and an aneuploidy induction test on *Salmonella cerevisiae* (about 7 % acetone) gave positive results. However, these two studies were considered not relevant on the basis of their poor quality and taking into account all the other negative genotoxicity results obtained with acetone, including results *in vivo* (see below).

In vivo data are available for four supporting substances: one saturated aliphatic secondary alcohol [FL-no: 02.079] and three saturated aliphatic ketones [FL-no: 07.017, 07.050 and 07.053], which

exhibited no genotoxic potential in the micronucleus cytogenetic assay at doses approaching the LD_{20} and the LD_{50} of the tested substances.

On the basis of available data from *in vitro* and *in vivo* tests on candidate and supporting substances, it can be concluded that the 43 candidate substances included in this group exhibit no genotoxic potential.

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

9. Conclusions

The FGE.07Rev2 includes the assessment of two additional flavouring substances [FL-no: 02.255 and 07.239] compared to FGE.07Rev1. Therefore, the present FGE.07Rev2 deals with 43 flavouring substances. These conclusions are an update of the previous versions.

The 43 candidate substances are saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain saturated carboxylic acids from chemical group 5.

Twenty-two of the 43 candidate substances possess a chiral centre [FL-no: 02.124, 02.142, 02.148, 02.177, 02.183, 02.190, 02.255, 07.157, 07.182, 07.185, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.676, 09.880 and 09.926], and two of the candidate substances possess two chiral centres [FL-no: 02.182 and 07.205]. For six of these substances [FL-no: 02.182, 02.190, 02.255, 09.676, 09.880 and 09.926] the stereoisomeric composition has not been specified.

Due to the presence and the position of double bonds, six of the 43 candidate substances can exist as geometrical isomers [FL-no: 02.255, 07.156, 07.236, 07.239, 09.386 and 09.880]. In three of these cases [FL-no: 07.156, 07.236 and 09.880] the stereoisomeric composition has not been specified.

Twenty-eight candidate substances [FL-no: 02.077, 02.124, 02.142, 02.148, 02.177, 02.182, 02.183, 02.190, 02.255, 07.084, 07.178, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880, 09.926] belong to structural class I and 15 candidate substances [FL-no: 07.072, 07.150, 07.156, 07.157, 07.158, 07.160, 07.162, 07.181, 07.185, 07.189, 07.199, 07.201, 07.205, 07.236, 07.182] belong to structural class II.

Thirty-nine of the flavouring substances in the present group of 43 flavouring substances have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, 43 candidate substances have European daily per capita intakes ranging from 0.0012 to 1.3 microgram, which are below the thresholds of concern for both structural class I and class II substances (1800 and 540 microgram/person/day, respectively).

On the basis of the reported annual production in Europe (MSDI approach), the combined intakes of the 28 of the candidate substances belonging to structural class I and of the 15 candidate substances belonging to structural class II would result in total intakes of 6 and 3.9 microgram/capita/day, respectively. These values are lower than the thresholds of concern for structural class I or class II substances. The total combined estimated levels of intake of the candidate and supporting substances is approximately 340 microgram/capita/day (without acetone and isopropanol) for

structural class I substances and 1080 microgram/capita/day for structural class II substances. This latter value does exceed the threshold of concern for the structural class. However, this level is not expected to saturate the detoxication reactions able to biotransform these compounds to innocuous products.

On the basis of available data from *in vitro* and *in vivo* tests on candidate and supporting substances, it can be concluded that the 43 candidate substances included in this group exhibit no genotoxic potential.

Forty-two of the 43 candidate substances would be expected to be metabolised to innocuous substances at the estimated levels of intake as flavouring substances. One candidate substance, 5-methylheptan-3-one [FL-no: 07.182], may be oxidised to a potential neurotoxic gamma-diketone. However, this metabolic path does not pose a safety concern at the estimated level of intake as a flavouring substance. Indeed, for this substance a NOAEL for neurotoxicity of 82 mg/kg bw/day was established in a subchronic study on adult male rats dosed with 0, 82, 410 and 820 mg/kg bw/day for 13 weeks, whereas the MSDI value is 0.32 microgram/capita/ day.

Otherwise it was noted, that where toxicity data were available on single flavouring substances, they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that on the basis of the default MSDI approach none of the 43 candidate substances would give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3900 microgram/person/day for the 28 candidate substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for three flavouring substances [FL-no: 07.084, 07.178 and 07.239]. The estimated intakes of the 15 candidate substances assigned to structural class II, based on the mTAMDI, range from 1500 to 1600 microgram/person/day, which are all above the threshold of concern for structural class II of 540 microgram/person/day. The three substances [FL-no: 07.084, 07.178 and 07.239], which have mTAMDI intake estimates below the threshold of concern for the structural class, are also expected to be metabolised to innocuous products.

Thus, for 40 of the 43 candidate substances considered in this Opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these 40 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the 43 candidate substances evaluated through the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including purity and identity for the materials of commerce have been provided for the 43 candidate substances.

Information on chirality has not been provided for six of the substances [FL-no: 02.182, 02.190, 02.255, 09.676, 09.880 and 09.926] and information on geometric isomerism has not been provided for three of the substances [FL-no: 07.156, 07.236 and 09.880]. Thus, the final evaluation of the

materials of commerce cannot be performed for eight substances [FL-no: 02.182, 02.190, 02.255, 07.156, 07.236, 09.676, 09.880 and 09.926], pending information on stereoisomerism.

The remaining 35 substances [FL-no: 02.077, 02.124, 02.142, 02.148, 02.177, 02.183, 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.162, 07.178, 07.181, 07.182, 07.185, 07.189, 07.199, 07.201, 07.205, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608 and 09.609] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 7, REVISION 2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 7, Revision 2

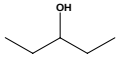
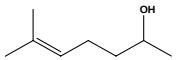
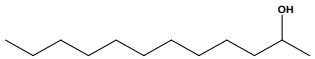
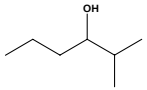
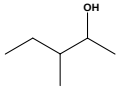
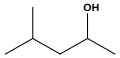
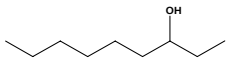
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. index 4) Spec. gravity 5)	Specification comments
02.077	Pentan-3-ol		2349 584-02-1	Liquid $C_5H_{12}O$ 88.15	Slightly soluble 1 ml in 1 ml	115 MS 98 %	1.407-1.413 0.815-0.822	
02.124	6-Methylhept-5-en-2-ol		10264 1569-60-4	Liquid $C_8H_{16}O$ 128.21	Slightly soluble 1 ml in 1 ml	77 (20 hPa) MS 95 %	1.447-1.453 0.848-0.854	Racemate.
02.142	3,3-Dimethylbutan-2-ol		464-07-3	Liquid $C_6H_{14}O$ 102.18	Slightly soluble 1 ml in 1 ml	120 MS 95 %	1.410-1.416 0.814-0.820	Racemate.
02.148	Dodecan-2-ol		11760 10203-28-8	Liquid $C_{12}H_{26}O$ 186.34	Insoluble 1 ml in 1 ml	129 (15 hPa) 19 MS 95 %	1.438-1.444 0.829-0.835	Racemate.
02.177	2-Methylhexan-3-ol		10266 617-29-8	Liquid $C_7H_{16}O$ 116.20	Slightly soluble 1 ml in 1 ml	144 MS 95 %	1.418-1.424 0.820-0.826	Racemate.
02.182	3-Methylpentan-2-ol 6)		10276 565-60-6	Liquid $C_6H_{14}O$ 102.18	Insoluble 1 ml in 1 ml	134 MS 95 %	1.415-1.421 0.827-0.833	Stereoisomers not specified by CASrn in Register.
02.183	4-Methylpentan-2-ol		10279 108-11-2	Liquid $C_6H_{14}O$ 102.18	Slightly soluble 1 ml in 1 ml	132 MS 99 %	1.407-1.414 0.802-0.808	Racemate.
02.190	Nonan-3-ol 6)		10290 624-51-1	Liquid $C_9H_{20}O$ 144.26	Practically insoluble or insoluble 1 ml in 1 ml	195 MS 95 %	1.425-1.431 0.818-0.824	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 7, Revision 2

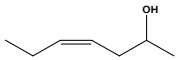
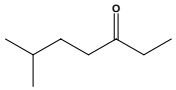
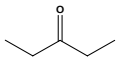
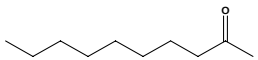
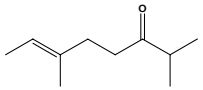
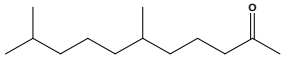
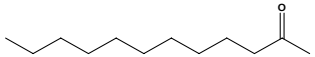
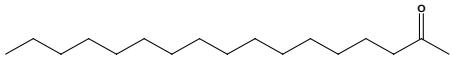
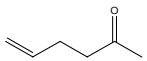
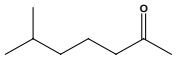
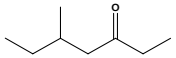
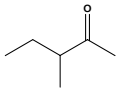
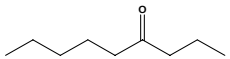
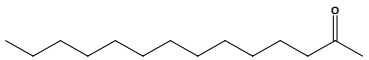
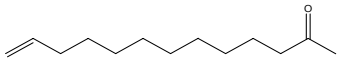
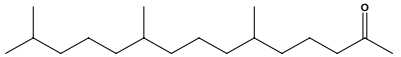
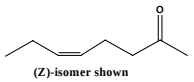
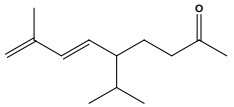
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. index 4) Spec. gravity 5)	Specification comments
02.255	(Z)-4-Hepten-2-ol 6)		66642-85-1	Liquid $C_7H_{14}O$ 114.19	Insoluble 1 ml in 1 ml	154 MS 91.77 %	1.433-1.453 0.832-0.852	Impurities: trans-4-hepten-2-ol (4.27 GC %), 2-heptanol (0.60 GC%), trans-3-hepten-2-ol (0.71 GC %), cis 3-hepten-2-ol (0.65 %).
07.072	6-Methylheptan-3-one		2143 624-42-0	Liquid $C_8H_{16}O$ 128.21	Insoluble 1 ml in 1 ml	162 MS 95 %	1.412-1.418 0.813-0.819	
07.084	Pentan-3-one		2350 96-22-0	Liquid $C_5H_{10}O$ 86.13	Partly soluble 1 ml in 1 ml	102 MS 99 %	1.389-1.395 0.812-0.818	
07.150	Decan-2-one		11055 693-54-9	Liquid $C_{10}H_{20}O$ 156.27	Insoluble 1 ml in 1 ml	210 MS 98 %	1.423-1.429 0.821-0.827	
07.156	2,6-Dimethyloct-6-en-3-one 6)	 (E)- isomer shown	2550-18-7	Liquid $C_{10}H_{18}O$ 154.25	Insoluble 1 ml in 1 ml	80 (13 hPa) NMR 95 %	1.442-1.448 0.823-0.829	(Z) or (E) isomer not specified by CASrn in Register.
07.157	6,10-Dimethylundecan-2-one		11068 1604-34-8	Liquid $C_{13}H_{26}O$ 198.35	Insoluble 1 ml in 1 ml	121 (16 hPa) MS 95 %	1.433-1.439 0.828-0.834	Racemate.
07.158	Dodecan-2-one		11069 6175-49-1	Liquid $C_{12}H_{24}O$ 184.32	Insoluble 1 ml in 1 ml	119 (13 hPa) 20 MS 99 %	1.431-1.437 0.825-0.835	
07.160	Heptadecan-2-one		11089 2922-51-2	Solid $C_{17}H_{34}O$ 254.46	Insoluble 1 ml in 1 ml	144 (1 hPa) 48 MS 95 %	n.a. n.a.	
07.162	Hex-5-en-2-one		109-49-9	Liquid $C_6H_{10}O$ 98.14	Slightly soluble 1 ml in 1 ml	128 MS 95 %	1.418-1.424 0.839-0.845	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 7, 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
07.178	3-Methylbutan-2-one		11131 563-80-4	Liquid $C_5H_{10}O$ 86.13	Slightly soluble 1 ml in 1 ml	94 MS 95 %	1.387-1.393 0.801-0.807	
07.181	6-Methylheptan-2-one		11146 928-68-7	Liquid $C_8H_{16}O$ 128.21	Insoluble 1 ml in 1 ml	167 MS 95 %	1.412-1.418 0.813-0.819	
07.182	5-Methylheptan-3-one		541-85-5	Liquid $C_8H_{16}O$ 128.21	Insoluble 1 ml in 1 ml	158 MS 95 %	1.418-1.424 0.816-0.824	Racemate.
07.185	3-Methylpentan-2-one		11157 565-61-7	Liquid $C_6H_{12}O$ 100.16	Insoluble 1 ml in 1 ml	117 MS 95 %	1.398-1.404 0.810-0.816	Racemate.
07.189	Nonan-4-one		11161 4485-09-0	Liquid $C_9H_{18}O$ 142.24	Insoluble 1 ml in 1 ml	188 MS 95 %	1.416-1.422 0.821-0.827	
07.199	Tetradecan-2-one		11192 2345-27-9	Solid $C_{14}H_{28}O$ 212.37	Insoluble 1 ml in 1 ml	146 (16 hPa) 33 MS 95 %	n.a. n.a.	
07.201	Tridec-12-en-2-one		60437-21-0	Liquid $C_{13}H_{24}O$ 196.33	Insoluble 1 ml in 1 ml	129 (13 hPa) NMR 95 %	1.441-1.447 0.815-0.821	
07.205	6,10,14-Trimethylpentadecan-2-one		11205 502-69-2	Liquid $C_{18}H_{36}O$ 268.48	Insoluble 1 ml in 1 ml	174 (13 hPa) MS 95 %	1.445-1.451 0.834-0.840	Racemate.
07.236	5-Octen-2-one 6)	 (Z)-isomer shown	11171 22610-86-2	Liquid $C_8H_{14}O$ 126.20	Practically insoluble or insoluble 1 ml in 1 ml	115 NMR 95 %	1.431-1.437 0.842-0.848	CASrn in Register refers to the (Z)-isomer.
07.239	[R-(E)]-5-Isopropyl-8-methylnona-6,8-dien-2-one		4331 2278-53-7	Liquid $C_{13}H_{22}O$ 194.31	Practically insoluble or insoluble 1 ml in 1 ml	238 MS 95 %	1.471-1.477 0.846-0.852	

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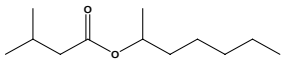
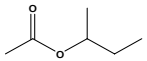
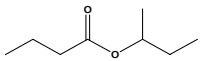
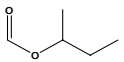
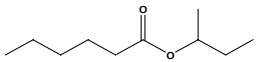
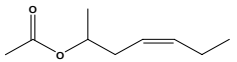
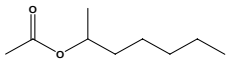
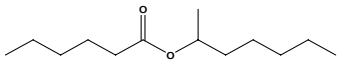
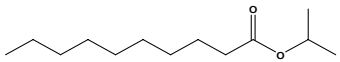
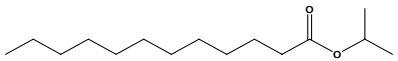
09.304	sec-Heptyl isovalerate		10806 238757-71-6	Liquid $C_{12}H_{24}O_2$ 200.32	Insoluble 1 ml in 1 ml	235 NMR 95 %	1.423-1.429 0.867-0.873	Racemate.
09.323	sec-Butyl acetate		10527 105-46-4	Liquid $C_6H_{12}O_2$ 116.16	Slightly soluble 1 ml in 1 ml	111 MS 95 %	1.385-1.391 0.867-0.873	Racemate.
09.325	sec-Butyl butyrate		10528 819-97-6	Liquid $C_8H_{16}O_2$ 144.21	Slightly soluble 1 ml in 1 ml	152 MS 95 %	1.399-1.405 0.858-0.864	Racemate.
09.328	sec-Butyl formate		10532 589-40-2	Liquid $C_5H_{10}O_2$ 102.13	Slightly soluble 1 ml in 1 ml	94 MS 95 %	1.386-1.392 0.877-0.883	Racemate.
09.332	sec-Butyl hexanoate		10533 820-00-8	Liquid $C_{10}H_{20}O_2$ 172.27	Insoluble 1 ml in 1 ml	82 (21 hPa) NMR 95 %	1.408-1.414 0.861-0.867	Racemate.
09.386	sec-Hept-4(cis)-enyl acetate		94088-33-2	Liquid $C_9H_{16}O_2$ 156.22	Insoluble 1 ml in 1 ml	185 MS 95 %	1.412-1.418 0.854-0.860	Racemate.
09.388	sec-Heptyl acetate		10802 5921-82-4	Liquid $C_9H_{18}O_2$ 158.24	Insoluble 1 ml in 1 ml	172 MS 95 %	1.406-1.412 0.862-0.868	Racemate.
09.391	sec-Heptyl hexanoate		10805 6624-58-4	Liquid $C_{13}H_{26}O_2$ 214.35	Insoluble 1 ml in 1 ml	126 (20 hPa) MS 95 %	1.421-1.427 0.851-0.857	Racemate.
09.604	Isopropyl decanoate		10730 2311-59-3	Liquid $C_{13}H_{26}O_2$ 214.35	Insoluble 1 ml in 1 ml	88 (3 hPa) MS 95 %	1.421-1.427 0.851-0.857	
09.605	Isopropyl dodecanoate		10233-13-3	Liquid $C_{15}H_{30}O_2$ 242.40	Insoluble 1 ml in 1 ml	105 (1 hPa) MS 95 %	1.427-1.433 0.851-0.857	

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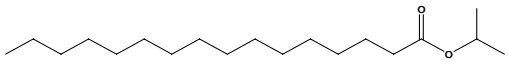
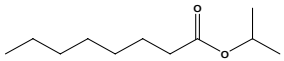
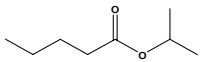
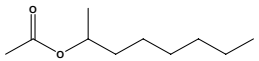
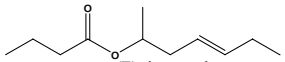
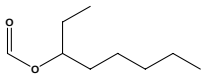
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. index 4) Spec. gravity 5)	Specification comments
09.606	Isopropyl hexadecanoate		10732 142-91-6	Liquid $C_{19}H_{38}O_2$ 298.51	Insoluble 1 ml in 1 ml	342 13 MS 95 %	1.433-1.439 0.852-0.858	
09.608	Isopropyl octanoate		10731 5458-59-3	Liquid $C_{11}H_{22}O_2$ 186.29	Insoluble 1 ml in 1 ml	124 (53 hPa) MS 95 %	1.414-1.420 0.853-0.859	
09.609	Isopropyl valerate		18362-97-5	Liquid $C_8H_{16}O_2$ 144.21	Insoluble 1 ml in 1 ml	165 MS 95 %	1.398-1.404 0.855-0.861	
09.676	sec-Octyl acetate 6)		10799 2051-50-5	Liquid $C_{10}H_{20}O_2$ 172.27	Practically insoluble or insoluble 1 ml in 1 ml	193 MS 95 %	1.409-1.415 0.857-0.863	
09.880	Hept-4-enyl-2 butyrate 6)	 (E)- isomer shown	233666-01-8	Liquid $C_{11}H_{20}O_2$ 184.28	Practically insoluble or insoluble 1 ml in 1 ml	224 MS 95 %	1.414-1.420 0.852-0.858	Neither (Z) or (E) isomer nor (R) or (S) enantiomer specified by CASrn in Register.
09.926	Octan-3-yl formate 6)		4009 84434-65-1	Liquid $C_9H_{18}O_2$ 158.24	Practically insoluble or insoluble 1 ml in 1 ml	71 (9 hPa) IR NMR MS 98 %	1.413-1.417 0.865-0.875	

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.

n.a.: not applicable.

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

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

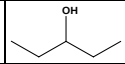
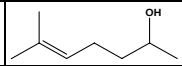
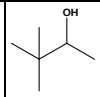
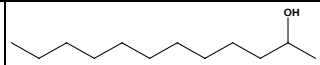
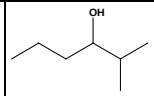
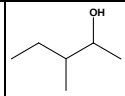
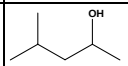
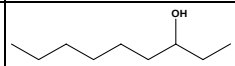
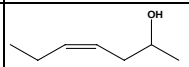
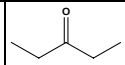
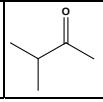
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.077	Pentan-3-ol		0.19	Class I A3: Intake below threshold	4)	6)	
02.124	6-Methylhept-5-en-2-ol		0.0061	Class I A3: Intake below threshold	4)	6)	
02.142	3,3-Dimethylbutan-2-ol		0.24	Class I A3: Intake below threshold	4)	6)	
02.148	Dodecan-2-ol		0.35	Class I A3: Intake below threshold	4)	6)	
02.177	2-Methylhexan-3-ol		0.12	Class I A3: Intake below threshold	4)	6)	
02.182	3-Methylpentan-2-ol		0.12	Class I A3: Intake below threshold	4)	7)	
02.183	4-Methylpentan-2-ol		0.0012	Class I A3: Intake below threshold	4)	6)	
02.190	Nonan-3-ol		0.011	Class I A3: Intake below threshold	4)	7)	
02.255	(Z)-4-Hepten-2-ol		0.03	Class I A3: Intake below threshold	4)	7)	
07.084	Pentan-3-one		0.24	Class I A3: Intake below threshold	4)	6)	
07.178	3-Methylbutan-2-one		0.073	Class I A3: Intake below threshold	4)	6)	

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

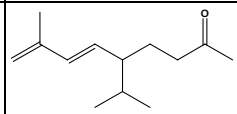
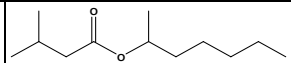
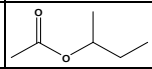
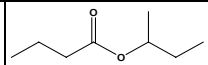
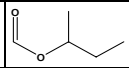
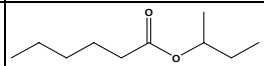
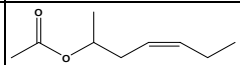
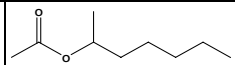
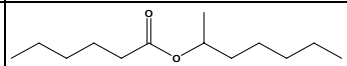
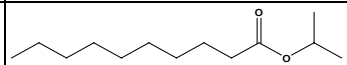
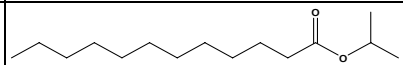
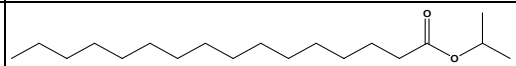
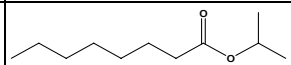
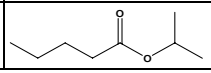
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
07.239 1840	[R-(E)]-5-Isopropyl-8-methylnona-6,8-dien-2-one		0.24	Class I A3: Intake below threshold	4)	6)	
09.304	sec-Heptyl isovalerate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.323	sec-Butyl acetate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.325	sec-Butyl butyrate		1.3	Class I A3: Intake below threshold	4)	6)	
09.328	sec-Butyl formate		0.12	Class I A3: Intake below threshold	4)	6)	
09.332	sec-Butyl hexanoate		0.024	Class I A3: Intake below threshold	4)	6)	
09.386	sec-Hept-4(cis)-enyl acetate		0.024	Class I A3: Intake below threshold	4)	6)	
09.388	sec-Heptyl acetate		0.12	Class I A3: Intake below threshold	4)	6)	
09.391	sec-Heptyl hexanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.604	Isopropyl decanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.605	Isopropyl dodecanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.606	Isopropyl hexadecanoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.608	Isopropyl octanoate		1.3	Class I A3: Intake below threshold	4)	6)	
09.609	Isopropyl valerate		0.012	Class I A3: Intake below threshold	4)	6)	

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

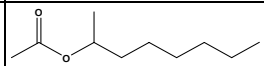
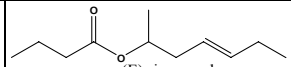
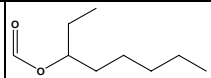
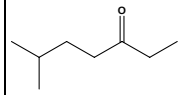
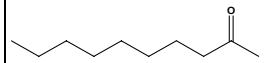
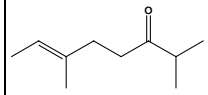
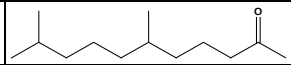
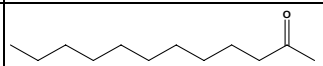
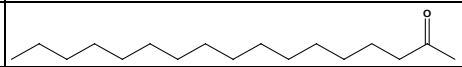
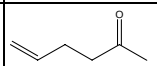
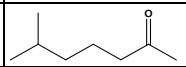
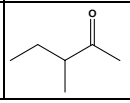
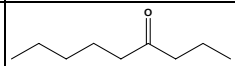
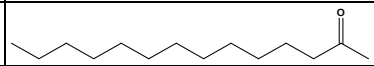
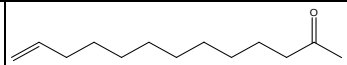
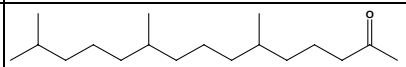
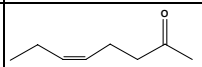
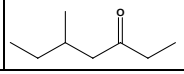
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.676	sec-Octyl acetate		0.011	Class I A3: Intake below threshold	4)	7)	
09.880	Hept-4-enyl-2 butyrate	 (E)- isomer shown	0.79	Class I A3: Intake below threshold	4)	7)	
09.926	Octan-3-yl formate		0.24	Class I A3: Intake below threshold	4)	7)	
07.072	6-Methylheptan-3-one		0.19	Class II A3: Intake below threshold	4)	6)	
07.150	Decan-2-one		0.52	Class II A3: Intake below threshold	4)	6)	
07.156	2,6-Dimethyloct-6-en-3-one	 (E)- isomer shown	0.0012	Class II A3: Intake below threshold	4)	7)	
07.157	6,10-Dimethylundecan-2-one		0.085	Class II A3: Intake below threshold	4)	6)	
07.158	Dodecan-2-one		0.73	Class II A3: Intake below threshold	4)	6)	
07.160	Heptadecan-2-one		0.12	Class II A3: Intake below threshold	4)	6)	
07.162	Hex-5-en-2-one		0.049	Class II A3: Intake below threshold	4)	6)	
07.181	6-Methylheptan-2-one		0.0012	Class II A3: Intake below threshold	4)	6)	
07.185	3-Methylpentan-2-one		1.2	Class II A3: Intake below threshold	4)	6)	

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
07.189	Nonan-4-one		0.52	Class II A3: Intake below threshold	4)	6)	
07.199	Tetradecan-2-one		0.073	Class II A3: Intake below threshold	4)	6)	
07.201	Tridec-12-en-2-one		0.024	Class II A3: Intake below threshold	4)	6)	
07.205	6,10,14-Trimethylpentadecan-2-one		0.0073	Class II A3: Intake below threshold	4)	6)	
07.236	5-Octen-2-one	 (Z)-isomer shown	0.0097	Class II A3: Intake below threshold	4)	7)	
07.182	5-Methylheptan-3-one		0.32	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	a)

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) NOAEL for neurotoxicity: 82 mg/kg bw/day; Adequate Margin of Safety.

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

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

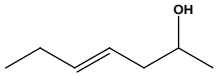
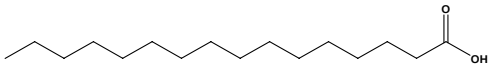
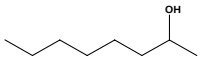
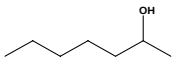
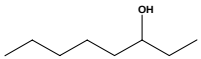
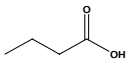
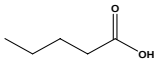
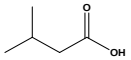
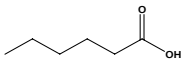
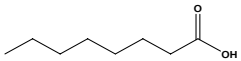
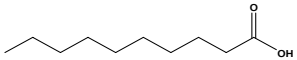
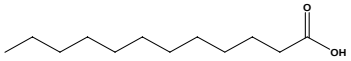
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	4-Hepten-2-ol	 (E)- isomer shown	Not evaluated as flavouring substance		Not in EU-Register.
	Hexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register.
02.022	Octan-2-ol 289		Category 1 a) No safety concern b) Category B c)	Class I A3: Intake below threshold	
02.045	Heptan-2-ol 284		Category 1 a) No safety concern b) Category B c)	Class I A3: Intake below threshold	
02.079	Isopropanol 277		Category 1 a) No safety concern b)	Class I A3: Intake above threshold, A4: Endogenous	
02.098	Octan-3-ol 291		Category 2 a) No safety concern b)	Class I A3: Intake below threshold	
02.121	Butan-2-ol		Category 1 a)	No evaluation	
08.001	Formic acid 79		Category 1 a) No safety concern d) Deleted c)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.005	Butyric acid 87		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.010	Octanoic acid 99		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.011	Decanoic acid 105		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
08.012	Dodecanoic acid 111		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	

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

2) No safety concern at estimated levels of intake.

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

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

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

a) (SCF, 1995).

b) (JECFA, 2000a).

c) (CoE, 1992).

d) (JECFA, 1999b).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

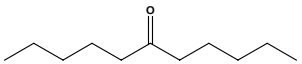
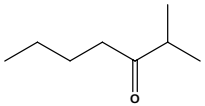
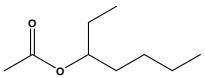
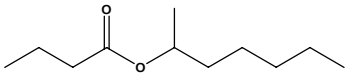
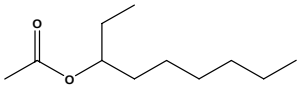
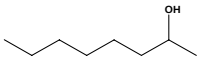
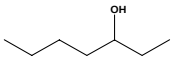
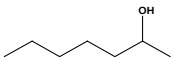
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	6-Undecanone		4022 927-49-1	1155 JECFA specification	3.0	No safety concern	Not in EU Register.
	2-Methylheptan-3-one		4000 13019-20-0	1156 JECFA specification	3.0	No safety concern	Not in EU Register.
	(+/-) Heptan-3-yl acetate		3980 5921-83-5	1143 JECFA specification	3.0	No safety concern	Not in EU Register.
	(+/-) Heptan-2-yl butyrate		3981 39026-94-3	1144 JECFA specification	3.0	No safety concern	Not in EU Register.
	(+/-) Nonan-3-yl acetate		4007 60826-15-5	1145 JECFA specification	3.0	No safety concern	Not in EU Register.
02.022	Octan-2-ol		2801 71 123-96-6	289 JECFA specification (JECFA, 1998b)	11	Category 1 a) No safety concern b) Category B c)	JECFA evaluated 2-octanol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
02.044	Heptan-3-ol		3547 544 589-82-2	286 JECFA specification (JECFA, 1998b)	0.12	Category 2 a) No safety concern b) Category B c)	JECFA evaluated 3-heptanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.045	Heptan-2-ol		3288 554 543-49-7	284 JECFA specification (JECFA, 1998b)	6.8	Category 1 a) No safety concern b) Category B c)	JECFA evaluated 2-heptanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.079	Isopropanol		2929 67-63-0	277 JECFA specification (JECFA, 1998b)	84000	Category 1 a) No safety concern b)	

Table 3: Supporting Substances Summary

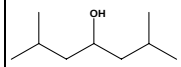
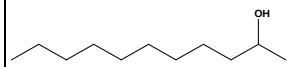
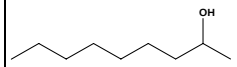
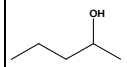
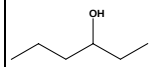
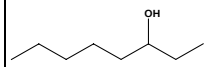
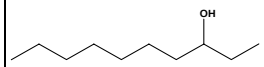
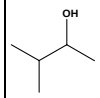
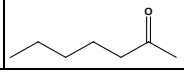
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.081	2,6-Dimethylheptan-4-ol		3140 11719 108-82-7	303 JECFA specification (JECFA, 1998b)	ND	Category 2 a) No safety concern b)	
02.086	Undecan-2-ol		3246 11826 1653-30-1	297 JECFA specification (JECFA, 1998b)	0.24	Category 1 a) No safety concern b)	JECFA evaluated 2-undecanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.087	Nonan-2-ol		3315 11803 628-99-9	293 JECFA specification (JECFA, 1998b)	0.61	Category 1 a) No safety concern b)	JECFA evaluated 2-nonanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.088	Pentan-2-ol		3316 11696 6032-29-7	280 JECFA specification (JECFA, 1998b)	5.4	Category 1 a) No safety concern b)	JECFA evaluated 2-pentanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.089	Hexan-3-ol		3351 11775 623-37-0	282 JECFA specification (JECFA, 1998b)	11	Category 2 a) No safety concern b)	JECFA evaluated 3-hexanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.098	Octan-3-ol		3581 11715 589-98-0	291 JECFA specification (JECFA, 1998b)	4.7	Category 2 a) No safety concern b)	JECFA evaluated 3-octanol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
02.103	Decan-3-ol		3605 10194 1565-81-7	295 JECFA specification (JECFA, 1998b)	ND	Category 2 a) No safety concern b)	JECFA evaluated 3-decanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
02.111	3-Methylbutan-2-ol		3703 598-75-4	300 JECFA specification (JECFA, 2000d)	0.49	Category 2 a) No safety concern b)	JECFA evaluated 3-methyl-2-butanol (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
07.002	Heptan-2-one		2544 136 110-43-0	283 JECFA specification (JECFA, 1998b)	96	Category 1 a) No safety concern b) Category A c)	

Table 3: Supporting Substances Summary

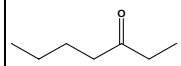
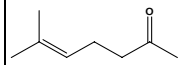
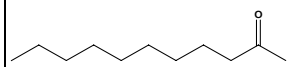
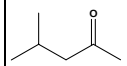
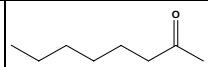
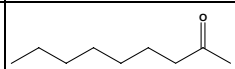
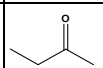
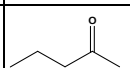
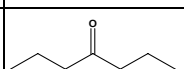
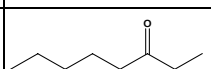
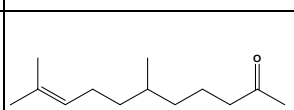
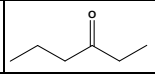
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.003	Heptan-3-one		2545 137 106-35-4	285 JECFA specification (JECFA, 1998b)	3.3	Category 2 a) No safety concern b) Category B c)	
07.015	6-Methylhept-5-en-2-one		2707 149 110-93-0	1120 JECFA specification (JECFA, 2002d)	100	No safety concern d) Category B c)	
07.016	Undecan-2-one		3093 150 112-12-9	296 JECFA specification (JECFA, 1998b)	330	Category 1 a) No safety concern b) Category A c)	
07.017	4-Methylpentan-2-one		2731 151 108-10-1	301 JECFA specification (JECFA, 1998b)	6.1	No safety concern b) Category B c)	
07.019	Octan-2-one		2802 153 111-13-7	288 JECFA specification (JECFA, 1998b)	93	Category 1 a) No safety concern b) Category A c)	
07.020	Nonan-2-one		2785 154 821-55-6	292 JECFA specification (JECFA, 1998b)	320	Category 1 a) No safety concern b) Category A c)	
07.050	Acetone		3326 737 67-64-1	139 JECFA specification (JECFA, 1998b)	6100	Category 1 a) No safety concern b) Category A c)	
07.053	Butan-2-one		2170 753 78-93-3	278 JECFA specification (JECFA, 1998b)	96	Category 1 a) No safety concern b) Category A c)	
07.054	Pentan-2-one		2842 754 107-87-9	279 JECFA specification (JECFA, 1998b)	120	Category 1 a) No safety concern b) Category A c)	
07.058	Heptan-4-one		2546 2034 123-19-3	287 JECFA specification (JECFA, 1998b)	1.9	Category 2 a) No safety concern b) Category B c)	
07.062	Octan-3-one		2803 2042 106-68-3	290 JECFA specification (JECFA, 1998b)	2.8	Category 2 a) No safety concern b) Category B c)	
07.069	Tetrahydro-pseudo-ionone		3059 2053 4433-36-7	1121 JECFA specification (JECFA, 2002d)	ND	No safety concern d) Category B c)	JECFA evaluated 3,4,5,6-tetrahydropseudoionone (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
07.096	Hexan-3-one		3290 11097 589-38-8	281 JECFA specification (JECFA, 1998b)	0.37	Category 2 a) No safety concern b)	

Table 3: Supporting Substances Summary

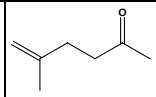
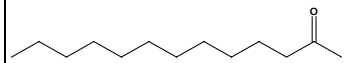
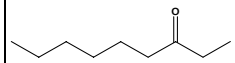
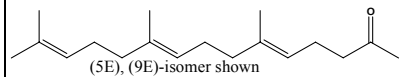
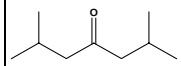
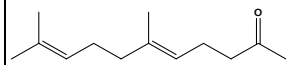
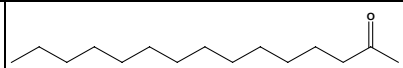
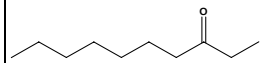
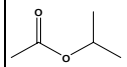
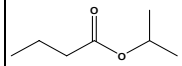
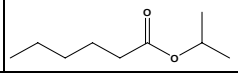
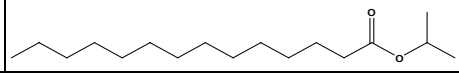
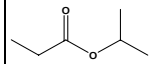
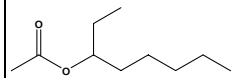
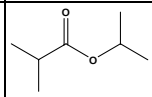
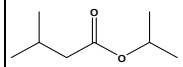
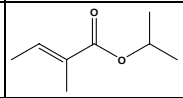
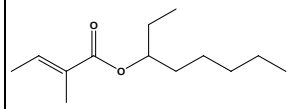
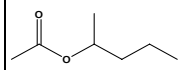
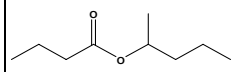
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
07.100	5-Methylhex-5-en-2-one		3365 11150 3240-09-3	1119 JECFA specification (JECFA, 2002d)	ND	No safety concern d)	
07.103	Tridecan-2-one		3388 11194 593-08-8	298 JECFA specification (JECFA, 2000d)	62	Category 1 a) No safety concern b)	
07.113	Nonan-3-one		3440 11160 925-78-0	294 JECFA specification (JECFA, 1998b)	0.12	Category 2 a) No safety concern b)	
07.114	6,10,14-Trimethylpentadeca-5,9,13-trien-2-one	 (5E), (9E)-isomer shown	3442 11206 762-29-8	1123 JECFA specification (JECFA, 2002d)	0.085	No safety concern d)	JECFA evaluated 2,6,10-trimethyl-2,6,10-pentadecatrien-14-one (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
07.122	2,6-Dimethylheptan-4-one		3537 11914 108-83-8	302 JECFA specification (JECFA, 1998b)	0.18	No safety concern b)	
07.123	Geranylacetone		3542 11088 3796-70-1	1122 JECFA specification (JECFA, 2002d)	41	No safety concern d)	JECFA evaluated 6,10-dimethyl-5,9-undecadien-2-one (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
07.137	Pentadecan-2-one		3724 11808 2345-28-0	299 JECFA specification (JECFA, 2000d)	18	Category 1 a) No safety concern b)	
07.151	Decan-3-one		3966 11056 928-80-3	1118 JECFA specification (JECFA, 2002d)	3.0	No safety concern d)	
09.003	Isopropyl acetate		2926 193 108-21-4	305 JECFA specification (JECFA, 1998b)	35	No safety concern b) Category A c)	No ADI allocated (JECFA, 1980a).
09.041	Isopropyl butyrate		2935 267 638-11-9	307 JECFA specification (JECFA, 1998b)	6.0	No safety concern b) Category A c)	
09.062	Isopropyl hexanoate		2950 312 2311-46-8	308 JECFA specification (JECFA, 2001c)	3.2	No safety concern b) Category A c)	

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
09.105	Isopropyl tetradecanoate		3556 386 110-27-0	311 JECFA specification (JECFA, 2000d)	19	No safety concern b) Category B c)	
09.123	Isopropyl propionate		2959 404 637-78-5	306 JECFA specification (JECFA, 2001c)	0.012	No safety concern b) Category A c)	
09.165	Isopropyl formate		2944 503 625-55-8	304 JECFA specification (JECFA, 2001c)	0.45	No safety concern b) Category A c)	
09.254	3-Octyl acetate		3583 2347 4864-61-3	313 JECFA specification (JECFA, 1998b)	0.61	No safety concern b) Category B c)	JECFA evaluated 3-octyl acetate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.415	Isopropyl isobutyrate		2937 290 617-50-5	309 JECFA specification (JECFA, 1998b)	0.49	No safety concern b) Category A c)	
09.450	Isopropyl isovalerate		2961 445 32665-23-9	310 JECFA specification (JECFA, 2002d)	0.24	No safety concern b) Category B c)	
09.513	Isopropyl 2-methylcrotonate		3229 10733 1733-25-1	312 JECFA specification (JECFA, 1998b)	0.012	No safety concern b)	JECFA evaluated isopropyl tiglate (CASrn 6284-46-4). CASrn in Register refers to (E)-isomer.
09.539	Oct-3-yl 2-methylcrotonate		3676 94133-92-3	448 JECFA specification (JECFA, 2001c)	0.012	No safety concern b)	JECFA evaluated 1-ethylhexyl tiglate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.657	1-Methylbutyl acetate		4012 10761 626-38-0	1146 JECFA specification (JECFA, 2002d)	2.9	No safety concern d)	JECFA evaluated 2-pentyl acetate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.658	1-Methylbutyl butyrate		3893 10763 60415-61-4	1142 JECFA specification (JECFA, 2002d)	ND	No safety concern d)	JECFA evaluated 2-pentyl buturate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.

ND) No intake data reported

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) (SCF, 1995).

b) (JECFA, 2000a).

c) (CoE, 1992).d) (JECFA, 2002c).

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.

Structural class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Structural class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Structural 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[6] (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[7] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

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

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

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

7 "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

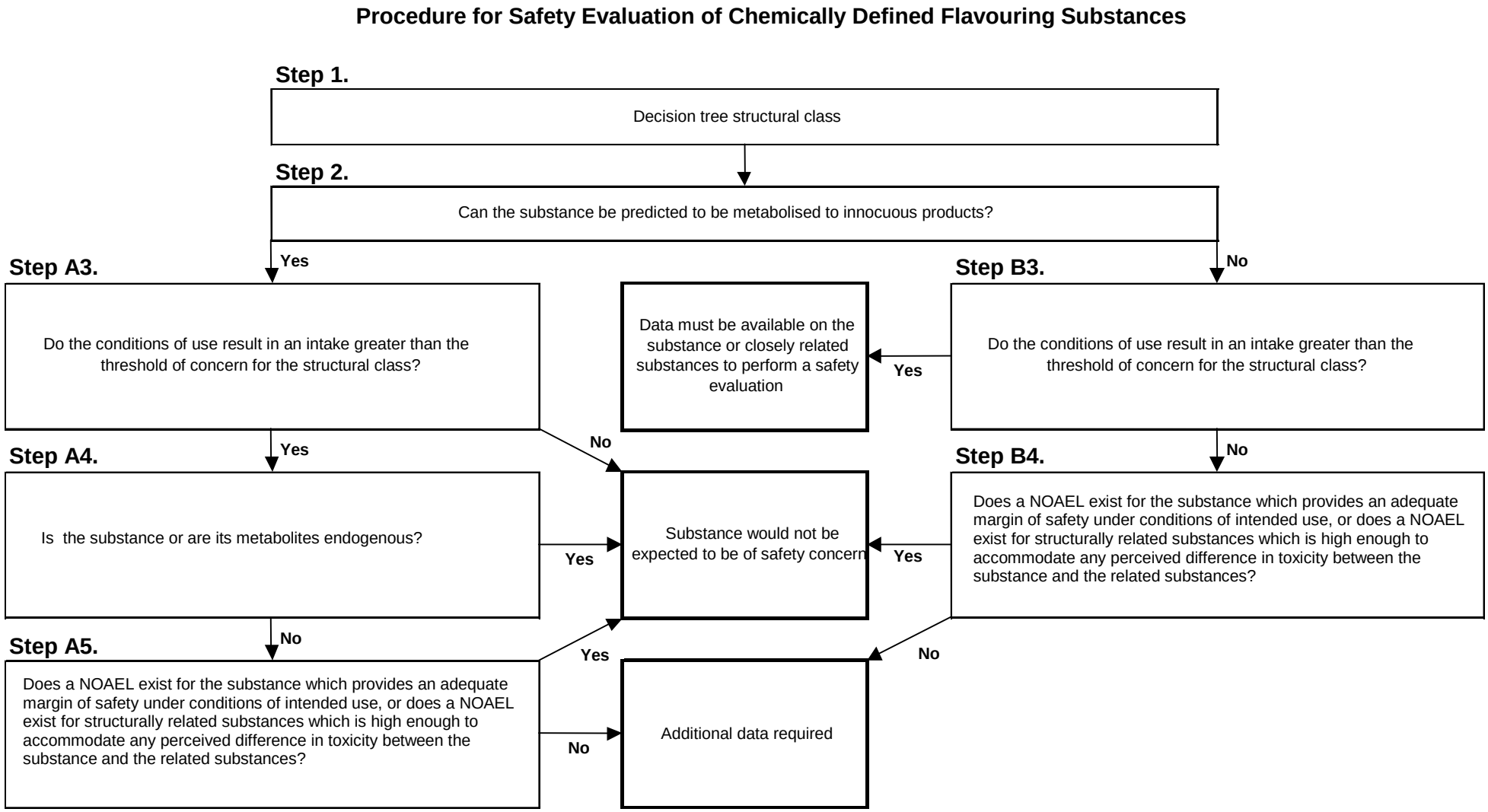


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 (MCE)
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 all 43 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.07Rev2 (EFFA, 2002b; EFFA, 2002f ; EFFA, 2007a; EFFA, 2007b; EFFA, 2007k; Flavour Industry, 2006p)

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.077	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.124	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.142	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.148	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.177	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.182	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.183	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.190	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
02.255	5 20	- -	10 50	- -	- -	10 60	- -	10 60	- -	- -	- -	- -	- -	5 20	2 10	10 40	- -	- -
07.072	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.084	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10

07.150	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.156	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	- -	3 15	2 10	4 20	5 25	2 10
07.157	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	5 25	2 10	4 20	- -	2 10
07.158	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.160	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.162	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.178	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.181	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.182	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.185	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.189	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.199	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.201	3 15	2 10	3 10	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.205	3 15	2 10	3 15	2 10	- -	- -	4 20	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
07.236	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
07.239	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	4 20	5 25	2 10
09.304	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.323	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.325	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.328	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	2 25
09.332	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.386	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.388	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.391	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.604	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.605	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.606	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.608	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	- -	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.609	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	- -	5 25
09.676	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.880	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25
09.926	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	10 50	20 100	5 25

II.2 mTAMDI Calculations

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

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

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

The 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		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms (MCE)	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 43 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2002b; EFFA, 2002f; EFFA, 2007a; EFFA, 2007b; EFFA, 2007k; Flavour Industry, 2006p). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.077	Pentan-3-ol	3900	Class I	1800
02.124	6-Methylhept-5-en-2-ol	3900	Class I	1800
02.142	3,3-Dimethylbutan-2-ol	3900	Class I	1800
02.148	Dodecan-2-ol	3900	Class I	1800
02.177	2-Methylhexan-3-ol	3900	Class I	1800
02.182	3-Methylpentan-2-ol	3900	Class I	1800
02.183	4-Methylpentan-2-ol	3900	Class I	1800
02.190	Nonan-3-ol	3900	Class I	1800
02.255	(Z)-4-Hepten-2-ol	2500	Class I	1800
07.084	Pentan-3-one	1600	Class I	1800
07.178	3-Methylbutan-2-one	1600	Class I	1800
07.239	[R-(E)]-5-Isopropyl-8-methylnona-6,8-dien-2-one	1600	Class I	1800
09.304	sec-Heptyl isovalerate	3900	Class I	1800
09.323	sec-Butyl acetate	3900	Class I	1800
09.325	sec-Butyl butyrate	3900	Class I	1800
09.328	sec-Butyl formate	3900	Class I	1800
09.332	sec-Butyl hexanoate	3900	Class I	1800
09.386	sec-Hept-4(cis)-enyl acetate	3900	Class I	1800
09.388	sec-Heptyl acetate	3900	Class I	1800
09.391	sec-Heptyl hexanoate	3900	Class I	1800
09.604	Isopropyl decanoate	3900	Class I	1800
09.605	Isopropyl dodecanoate	3900	Class I	1800
09.606	Isopropyl hexadecanoate	3900	Class I	1800
09.608	Isopropyl octanoate	3900	Class I	1800
09.609	Isopropyl valerate	3500	Class I	1800
09.676	sec-Octyl acetate	3900	Class I	1800
09.880	Hept-4-enyl-2 butyrate	3900	Class I	1800
09.926	Octan-3-yl formate	3900	Class I	1800
07.072	6-Methylheptan-3-one	1600	Class II	540
07.150	Decan-2-one	1600	Class II	540
07.156	2,6-Dimethyloct-6-en-3-one	1600	Class II	540
07.157	6,10-Dimethylundecan-2-one	1500	Class II	540
07.158	Dodecan-2-one	1600	Class II	540
07.160	Heptadecan-2-one	1600	Class II	540
07.162	Hex-5-en-2-one	1600	Class II	540
07.181	6-Methylheptan-2-one	1600	Class II	540
07.185	3-Methylpentan-2-one	1600	Class II	540
07.189	Nonan-4-one	1600	Class II	540
07.199	Tetradecan-2-one	1600	Class II	540
07.201	Tridec-12-en-2-one	1600	Class II	540
07.205	6,10,14-Trimethylpentadecan-2-one	1500	Class II	540
07.236	5-Octen-2-one	1600	Class II	540
07.182	5-Methylheptan-3-one	1600	Class II	540

ANNEX III: METABOLISM

III.1. General Information

The present flavouring group evaluation consists of 43 candidate substances of which seven are saturated aliphatic acyclic secondary alcohols [FL-no: 02.077, 02.142, 02.148, 02.177, 02.182, 02.183 and 02.190], two are unsaturated aliphatic secondary alcohol [FL-no: 02.124 and 02.255], 13 are saturated aliphatic ketones [FL-no: 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.178, 07.181, 07.182, 07.185, 07.189, 07.199 and 07.205], five are unsaturated aliphatic ketones [FL-no: 07.156, 07.162, 07.201, 07.236 and 07.239] of which three contain a terminal double bond [FL-no: 07.162, 07.201 and 07.239] and 16 are esters of aliphatic acyclic secondary alcohols and linear aliphatic carboxylic acids [FL-no: 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926].

The general metabolic reactions that the candidate substances may be expected to undergo, and which are discussed below, are one or several of the following:

- conjugation of secondary alcohols with glucuronic acid
- oxidation of secondary alcohols
- reduction of ketones
- oxidation of ketones
- oxidation of double bonds
- hydrolysis of esters
- oxidation of terminal double bonds.

A general discussion on the biotransformation of Saturated Aliphatic Acyclic Secondary Alcohols, Ketones, and Related Saturated and Unsaturated Esters may be found in the reports from the 51st and 59th meetings of JECFA (JECFA, 1999a; JECFA, 2002c). The discussions and conclusions related to these supporting substances essentially apply also to the candidate substances.

There is one candidate substance, 5-methylheptan-3-one [FL-no: 07.182], that may be oxidised to yield a neurotoxic gamma-diketone and therefore it may potentially give rise to concern.

III.2. Absorption

In general aliphatic secondary alcohols and ketones are expected to be rapidly absorbed in the gastrointestinal tract (JECFA, 1999a).

Peak blood levels were obtained 1 to 2 hours (h) after dosing when isopropanol was given orally to rats as well as when the same substance was administered intravenously to dogs (Lehman et al., 1945; Nordmann et al., 1973a). Peak blood levels were also obtained within 2 hours when 1- and 2-propanol, or 1- and 2-isobutanol were given orally to human volunteers together with ethanol (Bonte et al., 1981a).

In a pharmacokinetic experiment 2-butanol (2.2 ml/kg bw or 1776 mg/kg bw), 2-butanone (2.1 ml/kg bw or 1690 mg/kg bw) and 2,3-butanediol (0.68 ml/kg bw or 676 mg/kg bw), respectively, were administered orally in aqueous solutions to male Sprague-Dawley rats. Peak blood

concentrations after administration of 0.95 mg/l 2-butanone were detected after 4 hours (h) and declined to 0.07 mg/ml after 18 h. The concentrations of the metabolites 2,3-butanediol, 2-butanol and 3-hydroxy-2-butanone peaked at 0.26 mg/l, 0.033 mg/l and 0.027 mg/l at 18 h, 6 h and 8 h, respectively, after 2-butanone administration. Total AUC (Area Under the Curve) values for 2-butanone, 2,3-butanediol, 2-butanol and 3-hydroxy-2-butanone were 10.899±824, 3863±238, 414±38 and 382±38 mg h/l, respectively. Blood concentration after administration of 2-butanol peaked after 2 h at 0.59 mg/l and declined to 0.05 mg/l after 16 h. The blood concentrations of 2-butanone, 3-hydroxy-2-butanone and 2,3-butanediol rose to maximums after 8, 12 and 18 h and were 0.78, 0.04 and 0.21 mg/l, respectively. Total AUC values were 3254±258 mg h/l for 2-butanol, 9868±566 for 2-butanone, 443±93 for 3-hydroxy-2-butanone and 3167±503 mg h/l for 2,3-butanediol (Dietz et al., 1981).

Rats were administered 1 g/kg bw 2-pentanol, 3-pentanol and 3-methyl-2-butanol, via intraperitoneal injection. The alcohols were eliminated within 13 to 16 hours (Haggard et al., 1945).

III.3. Metabolism and Elimination

III.3.1. Secondary Alcohol

Oxidation and glucuronic acid conjugation

Secondary alcohols may undergo oxidation to the corresponding ketone. However, this reaction is generally unfavoured *in vivo*, since the alcohol is removed from the equilibrium by conjugation with glucuronic acid, which represents the major biotransformation pathway for secondary alcohols (Kasper & Henton, 1980; JECFA, 1999a). Glucuronidation is a Phase-II-reaction, which involves the transfer of glucuronic acid in an activated form to functional groups of the substrate: in this case to the hydroxyl groups of the molecules. This renders highly polar products, for which excretion is facilitated. The reaction is catalysed by UDP-glucuronyl transferase, which exists in several isoforms with different substrate specificities. The enzymes are located in the endoplasmatic reticulum, and are found in most tissues including the liver. The glucuronic acid conjugates are primarily excreted in the urine or bile, depending on the relative molecular mass and the animal species. For the candidate secondary alcohols, the urine is expected to be the main route of elimination.

III.3.2. Ketones

In addition to reduction and oxidation pathways, low molecular weight ketones (carbon chain length <5) may be excreted unchanged in expired air (Brown et al., 1987). In mammals, oral doses of volatile ketones or their corresponding alcohols are mainly eliminated as the ketone in expired air. Lower amounts are excreted in the urine (Schwartz, 1989; Haggard et al., 1945; Scopinaro et al., 1947).

In the rat, 2-pentanone in expired air was the major metabolite following administration of 2-pentanol by intraperitoneal injection. Lower amounts of 2-pentanol were also exhaled and both metabolites were detected in the urine (Haggard et al., 1945). Similarly, unchanged 2-pentanone administered orally to dogs has been identified in the expired air (Schwartz, 1989).

Reduction of ketones

In general, the major metabolic pathway for the detoxification and excretion of aliphatic ketones involves reduction of the ketone to the corresponding secondary alcohol with subsequent excretion

as conjugate of glucuronic acid. This reaction is reversible under physiologic conditions, but *in vivo* the secondary alcohols are removed from the equilibrium by conjugation to glucuronic acid, as is stated above, and the reaction proceeds to form further secondary alcohols (Felsted & Bachur, 1980; JECFA, 1999a). Reduction of aliphatic ketones is mediated by alcohol dehydrogenase and NADH/NADPH-dependent cytosolic carbonyl reductases (Bosron & Li, 1980). According to Felsted and Bachur (1980) the reaction catalysed by carbonyl reductase is stereoselective and favours formation of the (S)-enantiomer of the alcohol (Felsted & Bachur, 1980).

In studies limited to the identification of urinary glucuronide, relatively high single dose levels of a homologous series of aliphatic secondary alcohols and ketones were administered individually by gavage to rabbits. The urinary excretion of glucuronic acid conjugates was determined after 24 hours (Kamil et al., 1953a). The substances, dose levels and average urinary output of glucuronide (UGAC) were as follows

Substance	Dose (mg/kg bw)	UGAC (%)
2-pentanol	735	44.8
2-heptanone	950	41.0
2-heptanol	965	54.6
3-heptanol	965	61.9
2-octanol	1081	15.5

Oxidation of ketones

Ketones may also be metabolised *via* omega- or omega-1-oxidation. Participation in these pathways depends on chain length, position of the carbonyl function and dose (Dietz et al., 1981; Topping et al., 1994).

Short chain ketones (C < 5) that contain a carbonyl function at the C-2 may undergo oxidation of the terminal methyl group and subsequent oxidation to yield an alpha-ketocarboxylic acid. As intermediary metabolites alpha-ketoacids undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which may be completely metabolised in the fatty acid pathway and citric acid cycle. Alternatively, omega-oxidation may occur to yield a hydroxyketone, which may be further reduced to a diol, e.g. 2,3-butanediol from butanone, and excreted in the urine as a glucuronic acid conjugate.

Longer chain aliphatic ketones (carbon chain length ≥5) are primarily metabolised via reduction, but omega- and omega-1-oxidation are competing pathways at high concentrations (Dietz et al., 1981; Topping et al., 1994).

Studies with specific substances

4-Methylpentan-2-ol [candidate substance FL-no: 02.183] and 4-hydroxy-4-methylpentan-2-one were detected in serum after intraperitoneal injection of methyl-4-pentan-2-one to guinea pigs. The half-life and clearance times of 4-methylpentan-2-one were 66 minutes and 6 hours, respectively. 4-Hydroxy-4-methylpentan-2-one was the principal metabolite and was cleared in 16 hours. The concentration of 4-methylpentan-2-ol [FL-no: 02.183] was too low for quantification. 4-Methyl-pentan-2-one is metabolised by reduction of the carbonyl group to form the secondary alcohol, 4-methylpentan-2-ol [FL-no: 02.183], and by oxidation at the omega-1 carbon atom to form the hydroxylated ketone, 4-hydroxy-4-methylpentan-2-one (DiVincenzo et al., 1976).

Gamma-Diketone formation

Omega-1 oxidation of aliphatic ketones with special structural features may yield neurotoxic gamma-diketones. The metabolic pathway includes oxidation of the omega-1-carbon, first to a hydroxyketone and then to a diketone. The gamma-spacing of the carbonyl functions has been shown to be a prerequisite for neurotoxic effects, only ketones with this structural feature may yield the neurotoxic metabolites. One of the candidate substances, 5-methyl-3-heptanone [FL-no: 07.182], may potentially be oxidised to a gamma-diketone, 3-methyl-2,5-heptanedione.

Studies have shown that neurotoxicity of selected ketones is related to a common metabolic pathway leading to the formation of a gamma-diketone, which is the metabolite that produces neuropathy. The neurotoxic effects show a specific anatomic and morphological type of nerve degeneration characterised by large multifocal axonal swellings, referred to as ”giant axonal” neuropathy. Clinical symptomatology in humans includes bilaterally symmetrical paresthesia, ”pins and needles” feeling, and muscle weakness, primarily in arms and legs. Except for 2,5-heptanedione and 3,6-octanedione, all metabolic interconversions are oxidation of the omega-1-carbon, first to a hydroxyketone and then to a gamma-diketone. When the omega-carbon is oxidised in preference to the omega-1-carbon, no gamma-diketone is formed (Topping et al., 1994).

Induction of clear and typical signs of neurotoxicity in male rats dosed with 5-methyl-3-heptanone [FL-no: 07.182] in a subchronic study supported the hypothesis that a gamma-diketone may be formed as toxic metabolite. Adult male rats, 5 per group, were administered 5-methyl-3-heptanone [FL-no: 07.182] by gavage five days a week for 13 weeks at doses of 0, 82, 410 and 820 mg/kg bw/day. In addition to clinical observations, a Functional Observation Battery (FOB) was conducted. The result of the FOB clearly indicated peripheral neuropathy in the highest dose group and similar but less severe deficits were detected in the middle dose group. No functional defects were observed in the low-dose group. Gross examination showed no treatment related effects at any dose, but microscopic examination of sciatic and tibial nerves from the highest dose group revealed lesions typical of ”giant axonal” neuropathy. In the mid-dose group some changes were observed that were not necessarily diagnostic of ”giant axonal” neuropathy, but appeared to reflect reparative processes in the nerves and may as such have represented a borderline effect. Nerves from the low-dose group did not show any evidence of pathology attributable to treatment. The NOAEL for methyl-5-heptan-3-one was in this study considered to be 82 mg/kg bw/day (IBM Corp., 1989).

Data suggest that the neurotoxicity of the diketone decreases as chain length increases, possibly owing to steric hindrance. However, chain length may not be important to some materials, as in the case of 5-nonanone. Another factor modifying the neurotoxic potential of these substances is the number and size of substituent groups located between the gamma-spaced carbonyls. Single methyl groups on the carbons located between the carbonyl groups increase the potential neurotoxicity, whereas two methyl groups positioned on one of the methyl groups between the carbonyls eliminate neurotoxicity (Topping et al., 1994).

Among the supporting substances 3-heptanone [FL-no: 07.003] and 3-heptanol [FL-no: 02.044] are the only substances that may be metabolised to yield neurotoxic gamma-diketones (Topping et al., 1994). The neurotoxicity for these substances is observed only at high doses.

In a study reported as a meeting abstract, aliphatic ketones (hexane-2-one, pentane-3-one, heptane-3-one, 4-methyl-2-pentanone and 3,3-dimethyl-2-butanone) were administered in drinking water to female Wistar rats. It was concluded that administration of approximately 1 g/kg bw/day of hexane-2-one for 120 days produced muscle weakness, atrophy and peripheral neuropathy. None of the other ketones produced significant neurological alterations (Homan & Maronpot, 1978).

In an oral gavage study Crl rats, 2 per group, were given 3-heptanone [FL-no: 07.003] (0.25, 0.5, 1 or 2g /kg bw/day, for 5 days/week for 14 weeks. The highest dose-group (approaching the LD_{50} value in rats = 2760 mg/kg bw) was the only one developing treatment-related neuropathologic lesions of typical ”giant-axonal” type. No neuropathology was observed in the lower dose groups (O'Donoghue et al., 1984). This study determined that 3-heptanone has a low neurotoxic potential; however when its intake was combined with methyl ethyl ketone, neurotoxic effects were potentiated, by stimulating 3-heptanone metabolism to 2,5-heptandione, a neurotoxic gamma-diketone (O'Donoghue et al., 1984).

Oxidation of terminal double bonds

Three of the candidate substances, hex-5-en-2-one, tridec-12-en-2-one and ([R-(E)]-5-isopropyl-8-methylnona-6,8-dien-2-one [FL-no: 07.162, 07.201 and 07.239] have terminal double bonds. These double bonds may be oxidised to the corresponding epoxides. Epoxides are highly reactive molecules, due to the large strain associated with the three membered ring structure, and they react easily with nucleophilic sites of cellular macromolecules. For this reason, several aliphatic alkene-derived epoxides (e.g. ethylene, isoprene, butadiene and glycidol) have been demonstrated to be carcinogenic (Melnick, 2002). However, epoxides can be conjugated with glutathione by glutathione S-transferases or hydrolysed to diols by epoxide hydrolases. The latter two reactions can be considered to be detoxications. 1-Alkenes are metabolised by P450, through both double bond oxidation to the corresponding epoxide and allylic oxidation (Chiappe et al., 1998). The rates of the two reactions measured with different P450 isoforms indicate that epoxide formation is generally favoured (Chiappe et al., 1998). Therefore, due to the similar position of the double bond, it cannot be ruled out that, in addition to the above mentioned metabolic pathways for ketones, the three candidate substances [FL-no: 07.162, 07.201 and 07.239] may be, at least partially, biotransformed to an epoxide.

However, based on the low levels of intake of alkenones characterised by a carbonyl group in a distant position to the terminal double bond, it is expected that the detoxication reactions would not be saturated and would outweigh the rate of epoxide formation.

The presence of the terminal double bond in these three candidate substances is therefore not considered of concern because epoxides can be detoxicated by conjugation with glutathione or by epoxide hydrolase mediated hydrolysis.

Furthermore, based on genotoxicity data available for seven out of 48 flavouring substances with terminal double bonds from the Register (EC, 1999a; EC, 2004a) it is not indicated that a terminal double bond distal to a functional group is a structural alert for genotoxicity.

III.4. Ester Hydrolysis

The aliphatic esters among the candidate substances are expected to be hydrolysed to their component secondary alcohols and carboxylic acids. The carboxylesterase or esterase classes of enzymes, the most important of which are the beta-esterases, catalyse ester hydrolysis (Heymann, 1980). In mammals these enzymes occur within the body in most tissues including the gut lumen and intestinal wall, but predominate in the hepatocytes (Heymann, 1980). The wide range of tissue distribution and the multiplicity of esterases generally give rise to rapid hydrolysis of esters *in vivo*.

There are no hydrolysis studies on the candidate substances, but there are *in vitro* hydrolysis data for structurally related esters.

In vitro hydrolysis studies of esters have been performed with specific carboxylesterase isoenzymes isolated from pig and rat livers (Arndt & Krisch, 1973; Junge & Heymann, 1979). The isoenzyme I exhibits an increase in enzyme binding (lower Km) and maximum velocity (Vmax) as the carbon chain length of either the alcohol or carboxylic acid component of the substrate increases. It is also shown that different isoenzymes show great differences in the hydrolysis rates. Isoenzyme V had an optimum for the C5 compound, while this isoenzyme exhibited a minimum activity with the butyl and pentyl acetates. Results of *in vitro* studies indicate that the rate of hydrolysis of straight-chain esters is approximately 100 times faster than the rate of hydrolysis of branched-chain esters.

Incubation of isopropyl butanoate, isopropyl phenylacetate, isoamyl acetate and isoamyl phenylacetate with pancreatin produced 40, 50, 20 and 100 % hydrolysis respectively after 2 hours (Leegwater & Straten, 1974a; Grundschober, 1977). Also, isoamyl acetate incubated with intestinal mucosa homogenates obtained from pigs demonstrated complete hydrolysis (Leegwater & Straten, 1974b; Grundschober, 1977).

Esters formed from aliphatic secondary alcohols were hydrolysed to their corresponding alcohols and carboxylic acids when incubated with liver homogenates or small intestinal homogenates obtained from male Wistar albino rats, artificial gastric juice or artificial pancreatic juice with half-lives ranging from less than one second to several hours depending on the incubation medium (Gangolli & Shilling, 1968; Longland et al., 1977). Rat liver homogenates and small intestinal preparations were found to be much more efficient than artificial pancreatic juice for hydrolysis of a variety of aliphatic esters. Also, hydrolysis in simulated intestinal fluid with pancreatin was much faster than in simulated gastric juice (Longland et al., 1977).

The data on substances structurally related to the candidate substances indicate that hydrolysis is the major pathway for the candidate substances that are esters of secondary alcohols, and that they will be hydrolysed to their component alcohols and carboxylic acids within a relatively short time.

III.5. Conclusion

In conclusion, it may be anticipated that the seven saturated aliphatic acyclic secondary alcohols [FL-no: 02.077, 02.142, 02.148, 02.177, 02.182, 02.183 and 02.190], the two unsaturated aliphatic secondary alcohols [FL-no: 02.124 and 02.255], the 12 of the 13 saturated aliphatic ketones [FL-no: 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.178, 07.181, 07.185, 07.189, 07.199 and 07.205], the five unsaturated aliphatic ketone [FL-no: 07.156, 07.162, 07.201, 07.236 and 07.239] and the 16 esters of aliphatic acyclic secondary alcohols and linear aliphatic carboxylic acids [FL-no: 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926] may be expected to be metabolised to innocuous substances at the estimated levels of intake as flavouring substances.

One candidate substance, 5-methyl-3-heptanone [FL-no: 07.182], may be oxidised to a potentially neurotoxic gamma-diketone, 3-methyl-2,5-heptanedione.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for ten candidate substances of the present flavouring group from chemical group 5, and for 24 supporting substances evaluated by JECFA at the 51st and 59th meetings. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Table IV.1: Acute toxicity

Chemical Name [FL-no]	Species	Sex	LD50 (mg/kg bw)	Reference
(Acetone [07.050])	Rat	M	8452	(Smyth et al., 1970)
	Rat	NR	8930	(Smyth et al., 1969b)
	Rat	NR	9750	(FDA, 1975a)
	Rat	NR	6800	(Kimura et al., 1971a)
	Rat	NR	3465	(Kohli et al., 1967)
	Mouse	M	5250	(Tanii et al., 1986)
	Rabbit	NR	5300	(Krasavage et al., 1982)
(Isopropyl alcohol [02.079])	Rat	NR	5840	(Smyth & Carpenter, 1948)
	Rat	NR	5280	(Lehman & Chase, 1944)
	Rat	NR	5300	(Kimura et al., 1971a)
	Rat	NR	5330	(FDA, 1975a)
	Mouse	NR	5070	(FDA, 1975a)
	Rabbit	NR	5040	(Lehman & Chase, 1944)
	Rabbit	NR	7990	(Munch, 1972)
	Dog	NR	4830	(Lehman & Chase, 1944)
(2-Butanone [07.053])	Rat	M	5490	(Smyth et al., 1962)
	Rat	NR	2730	(Kimura et al., 1971a)
	Rat	NR	3980	(Union Carbide Corp., 1956)
	Rat	F	5525	(Pozzani et al., 1959)
	Mouse	M	3137	(Zakhari et al., 1977)
	Mouse	M	4050	(Tanii et al., 1986)
(2-Pentanone [07.054])	Rat	M	3730	(Smyth et al., 1962)

Table IV.1: Acute toxicity

Chemical Name [FL-no]	Species	Sex	LD50 (mg/kg bw)	Reference
	Mouse	M	2205	(Tanii et al., 1986)
(2-Pentanol [02.088])	Rabbit	NR	2820	(Munch, 1972)
Pentan-3-one [07.084]	Rat	NR	2900	(BASF, 1969)
	Rat	NR	2140	(Panson & Winek, 1980)
	Rat	NR	2140	(Eder et al., 1982a)
	Rat	NR	2140	(Kennedy & Graepel, 1991)
	Rat	NR	3100	(Ibatullina & Larionova, 1997)
Pentan-3-ol [02.077]	Rat	NR	1870	(Eder et al., 1982a)
(3-Hexanone [07.096])	Rat	NR	2727	(Carpenter et al., 1974)
(2-Heptanone [07.002])	Rat	M	1670	(Smyth et al., 1962)
	Mouse	M	2407	(Tanii et al., 1986)
	Mouse	NR	1088	(Schafer & Bowles, 1985)
	Mouse	NR	730	(Srepel & Akacic, 1962)
(2-Heptanol [02.045])	Rat	M, F	2580	(Eder et al., 1982a)
(3-Heptanone [07.003])	Rat	NR	2760	(Smyth et al., 1949)
(3-Heptanol [02.044])	Rat	NR	1870	(Smyth et al., 1951a)
(4-Heptanone [07.058])	Rat	NR	3049	(Carpenter et al., 1974)
(2-Octanone [07.019])	Rat	NR	>5000	(Katz et al., 1980)
	Mouse	M	3823	(Tanii et al., 1986)
	Mouse	NR	3870	(Tanii et al., 1986)
(2-Octanol [02.022])	Rat	NR	3200	(Patty et al., 1935)
(3-Octanone [07.062])	Rat	NR	5000	(Shelanski & Moldovan, 1973a)
(2-Nonanone [07.020])	Rat	NR	3200	(RTECS, 1975)
	Mouse	M	7992	(Tanii et al., 1986)
Decan-2-one [07.150]	Mouse	M	7936	(Tanii et al., 1986)
(2-Undecanone [07.016])	Mouse	NR	950	(Schafer & Bowles, 1985)
	Mouse	M	5460	(Tanii et al., 1986)
Methyl-3-butan-2-one [07.178]	Mouse	M	2572	(Tanii et al., 1986)
	Rat	NR	148	(Kennedy & Graepel, 1991)
(4-Methyl-2-pentanone [07.017])	Rat	NR	2080	(Smyth et al., 1951a)
	Mouse	M	2670	(Tanii et al., 1986)

Table IV.1: Acute toxicity

Chemical Name [FL-no]	Species	Sex	LD50 (mg/kg bw)	Reference
	Mouse	NR	1200	(McOmie & Anderson, 1949a)
Methyl-4-pentan-2-ol [02.183]	Rat	NR	2590	(Smyth et al., 1951a)
Methyl-4-pentan-2-ol [02.183] continued	Mouse	NR	1500	(McOmie & Anderson, 1949a)
Methyl-6-heptan-2-one [07.181]	Rat	NR	6700	(BASF, 1975)
Methyl-5-heptan-3-one [07.182]	Rat	NR	3500	(Kennedy & Graepel, 1991)
(2,6-Dimethyl-4-heptanone [07.122])	Rat	NR	5750	(Smyth et al., 1949)
	Mouse	NR	2800	(McOmie & Anderson, 1949a)
	Mouse	NR	1416	(RTECS, 1975)
Trimethyl-6,10,14-pentadecan-2-one [07.205]	Rat	NR	>2000	(BASF, 1988)
(6-Methyl-5-hepten-2-one [07.015])	Mouse	M, F	3609	(Colaianni, 1967)
	Rat	M, F	4100	(Keating, 1972a)
(3,4,5,6-Tetra-hydropseudoionone [07.069])	Mouse	M, F	5200	(Moreno, 1982a)
	Rat	M, F	>5000	(Moreno, 1977a)
(6,10-Dimethyl-5,9-undecadien-2-one [07.123])	Mouse	M, F	8650	(Moreno, 1976b)
	Rat	M, F	>6800	(Hofmann, 1978a)
(2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one [07.114])	Rat	M, F	>5000	(deGroot et al., 1974)
(Isopropyl formate [09.165])	Rat	NR	4300	(FDA, 1975a)
	Rabbit	NR	2500	(FDA, 1975a)
	Guinea Pig	NR	2700	(FDA, 1975a)
	Chicken	NR	2100	(FDA, 1975a)
(Isopropyl acetate [09.003])	Rat	M, F	6750	(Eder et al., 1982a)
	Rat	NR	3000	(FDA, 1975a)
	Rabbit	NR	6945	(Munch, 1972)
Isopropyl hexadecanoate [09.606]	Rat	M, F	>40000	(Food and Drug Research Laboratories, Inc., 1976a)
	Rat	M, F	>8000	(Kolmar Research Center, 1972)
	Rat	M, F	>64000	(Bio-Toxicology Laboratories, 1982)
	Rat	NR	>5000	(Moreno, 1978c)
Sec-Butyl formate [09.328]	Rat	NR	11300	(Union Carbide Corp., 1980)

NR: Not Reported

Subacute / subchronic / chronic toxicity data are available for two candidate substances and for ten supporting substances of the present flavouring group. They were evaluated at the 51st and 59th JECFA meetings. No carcinogenicity data are available. The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE, SUBCHRONIC, CHONIC AND CARCINOGENICITY STUDIES

Table IV.2: Subacute, subchronic, chronic and carcinogenicity studies

Chemical Name [FL-no]	Species;Sex No. per Group	Route	Dose levels (mg/kg/day)	Duration	NOAEL (mg/kg/day)	Reference	Comments
(Acetone [07.050])	Rat; M, F 10	Drinking water	0, 250, 500, 1000, 2000, 5000	13 weeks	1000 [1]	(Dietz, 1991)	3. NTP study.
	Mouse; M, F 10	Drinking water	0, 312.5, 625, 1250, 2500, 5000 (M) 0, 625, 1250, 2500, 5000, 12500 (F)	13 weeks	2500 [1]	(Dietz, 1991)	3. NTP study.
	Rat; M, F 30	Gavage	0, 100, 500, 2500	90 days	100	(Sonawane et al., 1986)	3. Meeting abstract.
	Rat; NR 3	Drinking water	1000	4 weeks	1000 [1, 2]	(Spencer et al., 1978)	3. Examinations were limited to specific neurotoxic effects. No other parameter was monitored.
(Isopropyl alcohol [02.079])	Human; M 8	Oral	0, 2.6, 6.4	6 weeks	6.4 [2]	(Wills et al., 1969)	3. Paper published in a peer reviewed journal.
	Rat; M 22	Drinking water	0, 870, 1280, 1680, 2520	12 weeks	870	(Pilegaard & Ladefoged, 1993)	3. Good quality study.
Pentan-3-one [07.084]	Rat; F 5	Drinking water	0, 1860	120 days	Not detected (<1860)	(Union Carbide Corp., 1977)	Good quality unpublished report. Focused on neurotoxic effect.
(2-Heptanone [07.002])	Rat; M, F 15	Gavage (dissolved in corn oil)	0, 20, 100, 500	13 weeks	20	(Gaunt et al., 1972a)	3. Good quality study- peer-reviewed journal.
	Rat: NR 5	Drinking Water	0, 500	12 weeks	500 [1, 2]	(Spencer et al., 1978)	3. Good quality study- peer-reviewed journal.
(3-Heptanone [07.003])	Rat; M 2	Gavage	0, 250, 500, 1000, 2000, 4000	14 weeks	1000	(O'Donoghue et al., 1984)	3. Good quality study- peer-reviewed journal.
	Rat; F NR	Drinking Water	1000	120 days	1000 [1]	(Homan & Maronpot, 1978)	3. Meeting abstract.
	Rat; F 5	Drinking water	0, 27	120 days	27[2]	(Union Carbide Corp., 1977)	Good quality unpublished report. Focused on neurotoxic effect.
(4-Heptanone [07.058])	Rat; M 8	Gavage	0, 1000	90 days	not detected (<1000)	(O'Donoghue & Krasavage, 1980)	3. Good quality unpublished report.
	Rat; M 3	Gavage (undiluted)	0, 1000, 2000, 4000	3 weeks	not detected (<1000)	(Krasavage & O'Donoghue, 1979)	3. Good quality unpublished report.

Table IV.2: Subacute, subchronic, chronic and carcinogenicity studies

Chemical Name [FL-no]	Species;Sex No. per Group	Route	Dose levels (mg/kg/day)	Duration	NOAEL (mg/kg/day)	Reference	Comments
(2-Nonanone [07.020])	Rat; M 3	Gavage (undiluted)	0, 1000, 2000, 4000	3 weeks	not detected (<1000)	(Krasavage & O'Donoghue, 1979)	3. Good quality unpublished report.
	Rat; M 8	Gavage	0, 2000	90 days	not detected (<2000)	(O'Donoghue & Krasavage, 1980)	3. Good quality unpublished report.
(4-Methyl-2-pentanone [07.017])	Rat; M, F 5	Drinking water	0, 1040	120 days	not detected (<1040)	(Union Carbide Corp., 1977)	Good quality unpublished report. Focused on neurotoxic effect.
	Rat; F NR	Drinking water	1000	120 days	1000 [2]	(Homan & Maronpot, 1978)	3. Meeting abstract.
Methyl-5-heptan-3-one [07.182]	Rat; M 5	Gavage (in distilled water)	82, 410, 820 mg/kg bw/day	13 weeks (5 days/week)	82	(IBM Corp., 1989)	Good quality unpublished Report -submitted to EPA.
(2,6-Dimethyl-4-heptanone [07.122])	Rat; M 8	Gavage	0, 2000	90 days	not detected (<2000)	(O'Donoghue & Krasavage, 1980)	3. Good quality unpublished report.
(5-Methyl-5-hexen-2-one [07.100])	Rat; M, F 5	Diet	0, 10	14 days	10 [2]	(Gill & Van Miller, 1987a)	4. GLP study-unpublished report.
(2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one [07.114])	Rat; M, F 5	Oral (gavage in maize oil)	0, 0.35, 3.5	14 days	.3.5	(deGroot et al., 1974)	4. TNO Unpublished Report.

NR = sex not reported; M = Male; F = Female.

1. Concentrations converted to mg/kg bw/day using conversion table for test chemical treatment doses used in PAFA (FDA, 1993).

2.This study was performed at a single dose level that produced no adverse effects. Therefore, this dose level is not a true NOEL, but is the highest dose tested that produced no adverse effects. The actual NOEL would be higher.

3. Summarised by JECFA, 51st meeting (JECFA, 1999a).

4. Summarised by JECFA 59th meeting (JECFA, 2003a).

Developmental and reproductive toxicity data are only available for one candidate substances of the present flavouring group evaluation from chemical group 5 and for one supporting substance evaluated at the 51st JECFA meeting. Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical name	Study type/duration	Species/sex No/group	Route	NOAEL mg/kg/day including information on possible maternal toxicity	Reference	Comments
(Isopropyl alcohol [02.079])	Reproductive Toxicity: 2 generations with 10 weeks of dosing prior to mating	Rat; M, F 4; 60	Gavage	500	(Bevan et al., 1995)	1. EPA Guideline compliance.
	Developmental Toxicity: Gestation days 6-15	Rat; F 4; 25	Gavage	400 (maternal) 400 (foetal)	(Tyl et al., 1994)	1. EPA Guideline compliance.
	Developmental Toxicity: Gestation days 6-18	Rabbit; F 4; 15	Gavage	240 (maternal) 480 (foetal)	(Tyl et al., 1994)	1. EPA Guideline compliance.
Pentan-3-one [07.084]	Fertility Screen: 28 daily doses with mating starting on day 10	Mouse; F 2; 8	I.p.	50	(Carlson et al., 1975)	Few details given in the paper.

M = Male; F = Female.

1. Summarised by JECFA, 51st meeting (JECFA, 1999a).

In vitro mutagenicity/genotoxicity data are available for seven candidate substances of the present flavouring group evaluation from chemical group 5 and for nine supporting substances evaluated at the 51st and 59th JECFA meetings. Supporting substances are listed in brackets.

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

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

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
(Acetone [07.050])	Rec assay	*B. subtilis*	NR	Negative [1]	(Kawachi et al., 1980a)	8
	Rec assay	*B. subtilis*	NR	Negative	(Ishizaki et al., 1979)	8
	Ames test	*S. typhimurium* TA100	0.1 to 1000 µg/plate	Negative	(Rapson et al., 1980)	8
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	174 µg/plate	Negative [1]	(Florin et al., 1980)	8
	Ames test	*S. typhimurium* TA98, TA100	NR	Negative [1]	(Kawachi et al., 1980a)	8
	Ames test [2]	*S. typhimurium* TA98, TA100	30 µl/plate	Negative [4]	(Yamaguchi, 1985)	8
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	Up to 10000 µg/plate	Negative [1]	(McCann et al., 1975)	8
	Ames test [2]	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	Up to 10000 µg/plate	Negative [1]	(Zeiger et al., 1992)	8
	Ames test	*S. typhimurium* TA100	500 µg/plate	Negative [1]	(Yamaguchi, 1982)	8
	Ames test	*S. typhimurium* TA97, TA98, TA100	20 to 40 µg	Negative [1]	(Azizan & Blevins, 1995)	8
	Sister chromatid exchange	Human embryo fibroblasts	NR	Negative [4]	(Kawachi et al., 1980a)	8
	Sister chromatid exchange	Hamster lung fibroblasts	NR	Negative [4]	(Kawachi et al., 1980a)	8
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 10 µg/ml	Negative	(Sasaki et al., 1980)	8
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 5020 µg/ml	Negative [1]	(Loveday et al., 1990)	8
	Sister chromatid exchange	Diploid human fibroblasts	5 µg/ml	Negative	(Sasaki et al., 1980)	8
	Sister chromatid exchange	Human lymphocytes	395 µg/ml	Negative	(Norppa et al., 1983)	8
	Sister chromatid exchange	Human lymphocytes	0.1 to 1 mM	Negative	(Zarani et al., 1999)	8
	Chromosomal aberrations	Chinese hamster ovary cells	Up to 5020 µg/ml	Negative [1]	(Loveday et al., 1990)	8
	Chromosomal aberrations	Hamster lung fibroblasts	NR	Positive [4]	(Kawachi et al., 1980a)	8
	Aneuploidy induction	*S. cerevisiae*	6.98-7.83 %	Positive [4]	(Zimmermann et al., 1985a)	10
(Isopropyl alcohol [02.079])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	174 µg/plate	Negative [1]	(Florin et al., 1980)	8
	Ames test [2]	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. coli* WP2uvrA	5 to 5000 µg/plate	Negative [1]	(Shimizu et al., 1985)	8
	Ames test [2]	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Up to 10 mg/plate [5]	Negative [1]	(Zeiger et al., 1992)	8
	Forward mutation	Chinese hamster ovary cells [6]	0.5 to 5.0 mg/ml	Negative [1]	(CMA, 1990)	8
	Forward mutation	Chinese hamster ovary cells [6]	0.5 to 5.0 mg/ml	Negative [1]	(Kapp et al., 1993a)	8
(2-Butanone [07.053])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10000 µg/plate	Negative [1]	(Douglas et al., 1980)	8
	Ames test	*S. typhimurium* TA102, TA104	1 mg/plate	Negative	(Marnett et al., 1985a)	8
(2-Butanone [07.053]) continued	Ames test [2]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 to 5000 µg/plate	Negative [1]	(Shimizu et al., 1985)	8
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.04 to 26 µg/plate	Negative [1]	(O'Donoghue et al., 1988)	8

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

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
	Ames test [2]	*S. typhimurium* TA97, TA98, TA100, TA104, TA1535, TA1537	Up to 10000 µg/plate	Negative [1]	(Zeiger et al., 1992)	8
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative [4]	(Müller et al., 1993)	8
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2uvrA	4000 µg/plate	Negative	(Brooks et al., 1988)	8
	Gene conversion	*S. cerevisiae*	5 mg/ml	Negative [1]	(Brooks et al., 1988)	8
	Forward mutation	L5178Y/TL+/- mouse lymphoma cells	0.67 to 12 µg/ml	Negative [1]	(O'Donoghue et al., 1988)	8
	Unscheduled DNA synthesis	Human lymphocytes	0.72 mg/ml	Negative [1]	(Perocco et al., 1983)	8
	Unscheduled DNA synthesis	Rat hepatocytes	7.2 to 360 mg/ml	Negative	(O'Donoghue et al., 1988)	8
	Chromosomal aberrations	Rat hepatocytes	1000 µg/ml	Negative	(Brooks et al., 1988)	8
	Chromosomal aberrations	Chinese hamster ovary cells	1000 µg/ml	Negative [1]	(Brooks et al., 1988)	8
	Cell transformation assay[1]	BALB/3T3 cells (clone A31-1)	6-18 µl/ml	Negative	(O'Donoghue et al., 1988)	
	Aneuploidy induction	*S. cerevisiae*	3.38%	Positive [4]	(Zimmermann et al., 1985a)	10
Pentan-3-one [07.084]	Aneuploidy induction	*S. cerevisiae*	1.48%	Positive [4]	(Zimmermann et al., 1985a)	10
Pentan-3-ol [02.077]	Chromosomal aberrations	Chinese hamster ovary cells	0.5 to 10%	Negative [1]	(Abbondandolo et al., 1980)	
	Forward mutation	*S. pombe*	0.5 to 10%	Negative [1]	(Abbondandolo et al., 1980)	
(2-Heptanone [07.002])	Unscheduled DNA synthesis	Rat hepatocytes	1000 ppm	Negative	(Barber et al., 1999)	
Methyl-3-butan-2-one [07.178]	Aneuploidy induction	*S. cerevisiae*	1.23 to 1.36%	Negative [4]	(Zimmermann et al., 1985a)	10
	Aneuploidy induction	*S. cerevisiae*	0.84 to 1.23%	Negative [4]	(Zimmermann et al., 1985a)	10
(4-Methyl-2-pentanone [07.017])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.03 to 3 mg/plate	Negative [1]	(O'Donoghue et al., 1988)	8
	Ames test [2]	*S. typhimurium* TA97, TA98, TA100, TA1535	Up to 6667 µg/plate	Negative [1]	(Zeiger et al., 1992)	8
	Ames test	*E. coli* WP2uvrA	8000 µg/plate	Negative [4]	(Brooks et al., 1988)	8
	Gene conversion	*S. cerevisiae*	5 mg/ml	Negative [1]	(Brooks et al., 1988)	8
	Forward mutation	L5178Y/TL+/- mouse lymphoma cells	0.26 to 4.2 µg/ml	Negative [1]	(O'Donoghue et al., 1988)	8
	Unscheduled DNA synthesis	Rat hepatocytes	8 to 80 µg/ml	Negative	(O'Donoghue et al., 1988)	8
	Chromosomal aberrations	Rat hepatocytes	1000 µg/ml	Negative	(Brooks et al., 1988)	8
	Cell transformation assay[1]	BALB/3T3 cells (clone A31-1)	1-7µl/ml	Negative	(O'Donoghue et al., 1988)	
	Chromosomal aberrations	Chinese hamster ovary cells	1000 µg/ml	Negative [1]	(Brooks et al., 1988)	8
Methyl-4-pentan-2-ol [02.183]	Ames test [2]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2uvrA	5000 µg	Negative [1]	(Shimizu et al., 1985)	
Methyl-6-heptan-2-one [07.181]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	5000 µg/plate	Negative [1]	(BASF, 1989a)	
(2,6-Dimethyl-4-heptanone [07.122])	Ames test [2]	*S. typhimurium* TA98, TA100, TA1535, TA1537	1 to 333 µg/plate	Negative [1]	(Mortelmans et al., 1986)	8
Trimethyl-6,10,14-pentadecan-2-one [07.205]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	5000 µg/plate	Negative [1]	(BASF, 1989b)	
(6-Methyl-5-hepten-2-one [07.015])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	380 µg/plate	Negative [1]	(Florin et al., 1980)	9
(Isopropyl acetate [09.003])	Ames test [2]	*S. typhimurium* TA97, TA98, TA100, TA1537, TA1538	Up to 10 mg/plate	Negative [1]	(Zeiger et al., 1992)	8

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

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
(Isopropyl myristate [09.105])	Ames test [7]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	50 µg/plate	Negative [1]	(Blevins & Taylor, 1982)	8
Isopropyl hexadecanoate [09.606]	Ames test [7]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	50 µg/plate	Negative [1]	(Blevins & Taylor, 1982)	

1. Assay performed with and without metabolic activation.

2. Modified Ames (Pre-incubation) protocol.

3. Assay performed with S9 metabolic activation.

4. Assay performed without S9 metabolic activation.

5. Maximum non-toxic dose.

6. HGPRT locus.

7. Spot test.

8. Summarised by JECFA, 51st meeting (JECFA, 1999a).

9. Summarised by JECFA 59th meeting (JECFA, 2003a).

10. Unusual experimental protocol for detection of aneuploidy, which can be considered a threshold effect not mediated by a direct interaction with DNA. Positive results were obtained at concentrations approaching cytotoxic levels and are very likely due to the presence of technical artefacts (low temperature treatment inducing tubulin dissociation). Indeed, absence of effect was recorded when the ice treatment was skipped. – The limited relevance of fungal systems together with the uncertain quality of these results make questionable their extrapolation to the in vivo situation in humans.

In vivo mutagenicity / genotoxicity data available for four supporting substances evaluated at the 51st and 59th JECFA meetings.

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

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Isopropyl alcohol [02.079])	Micronucleus test	ICR Mouse (15M & 15F)	i.p. injection in 0.9% NaCl	350-2500 mg/kg	Negative	(Kapp et al., 1993a)	1
(Acetone [07.050])	Micronucleus test	Chinese hamster (5M & 5F)	i.p. injection in corn oil	865 mg/kg	Negative	(Basler, 1986)	1
(2-Butanone [07.053])	Micronucleus test	CD-1 mice (5M & 5F)	i.p. injection in corn oil	LD20 (1.96 ml/kg)	Negative	(O'Donoghue et al., 1988)	1
	Micronucleus test	Chinese hamster (5M & 5F)	i.p. injection in corn oil	411mg/kg	Negative	(Basler, 1986)	1
(4-Methyl-2-pentanone [07.017])	Micronucleus test	CD-1 mice (5M & 5F)	i.p. injection in corn oil	LD20 (0.73 ml/kg)	Negative	(Basler, 1986)	1

1. Summarised by JECFA, 51st meeting (JECFA, 1999a).

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ABBREVIATIONS

AUC	Area under curve
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
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)
FOB	Functional observational battery
GLP	Good Laboratory Practise
ID	Identity
IOFI	International Organization of the Flavour Industry
Ip	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
Km	Concentration of substrate that leads to half-maximal velocity
LD_{50}	Lethal Dose, 50%; Median lethal dose
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NADH	Nicotinamide adenine dinucleotide (NAD^+), reduced form (NADH)
NADPH	Nicotinamide adenine dinucleotide phosphate ($NADP^+$), reduced form (NADPH)
NMR	Nuclear magnetic resonance

No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
US EPA	United States Environmental Protection Agency
Vmax	Maximum velocity
WHO	World Health Organisation