EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2):

Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8.[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 32 flavouring substances in the Flavouring Group Evaluation 18, Revision 2, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that 28 substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For the remaining four substances [FL-no: 02.146, 02.185, 02.191 and 09.669] no appropriate NOAEL was available and additional data are required. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for six substances information is lacking.

1 On request from the Commission, Question No EFSA-Q-2010-01132, adopted on 30 September 2010.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) 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 32 flavouring substances in the Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 32 flavouring substances belong to chemical groups 6 and 8, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation deals with 32 saturated and unsaturated aliphatic acyclic and alicyclic tertiary alcohols, aromatic tertiary alcohols and their esters. Based on their structures, the candidate substances can be subdivided into 8 subgroups.

Nineteen of the 32 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-no: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.191, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For five of these substances [FL-no: 02.146, 02.147, 02.168, 02.191 and 02.197] the stereoisomeric composition has not been specified sufficiently. For four of these five substances [FL-no: 02.147, 02.168, 02.191 and 02.197] the stereoisomeric composition of the mixture has not been specified.

Twenty of the 32 candidate substances are classified into structural class I, 11 candidate substances are classified into structural class II and one is classified into structural class III according to the decision tree approach.

Twenty-four out of the 32 candidate substances have been reported to occur 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 Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

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

2-Methylpropan-2-ol [FL-no: 02.052] provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence and the lack of structural alerts for genotoxicity for this substance and its metabolites do not raise concern for *in vivo* genotoxicity. For the other substances in this group the available data considered valid do not give rise to any safety concerns with respect to genotoxicity.

Twenty-eight of the candidate substances are anticipated to be metabolised to innocuous products. For the remaining four candidate substances no metabolism data are available and therefore they cannot be predicted to be metabolised to innocuous products. No appropriate NOAEL was available for these

four candidate substances [FL-no: 02.146, 02.185, 02.191 and 09.669] or for the supporting substances. Therefore, additional data are required for these four candidate substances.

It is considered that, on the basis of the default MSDI approach, 28 of the 32 candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. For the remaining four substances [FL-no: 02.146, 02.185, 02.191 and 09.669], no appropriate NOAEL was available or for the supporting substances. Therefore, additional data are required for these four candidate substances.

The mTAMDI values for the 20 candidate substances from structural class I range from 3900 to 14000 microgram/person/day. For 10 of the 11 candidate substances from structural class II, the mTAMDI values are 3900 microgram/person/day for each of nine candidate substances, and 1600 microgram/person/day for one candidate substance. For one candidate substance [FL-no: 02.146] from structural class II no data on use and use levels are provided. For the one candidate substance from structural class III the mTAMDI is 5700 microgram/person/day. Accordingly, the estimated intakes for the 31 candidate substances for which use levels are provided are above the thresholds of concern for their structural classes. Therefore, for these 31 substances and the one substance for which no use and use levels have been provided 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 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for the 32 candidate substances. However, information on the stereoisomeric composition has not been specified sufficiently for five substances [FL-no: 02.146, 02.147, 02.168, 02.191 and 02.197]. In addition, the composition of the mixture of [FL-no: 02.129] is missing and for [FL-no: 02.129 and 02.146] are other information on specification lacking. Thus, the final evaluation of the materials of commerce cannot be performed for six substances [FL-no: 02.129, 02.146, 02.147, 02.168, 02.191 and 02.197], pending further information.

In conclusion, for four flavouring substances [FL-no: 02.146, 02.185, 02.191 and 09.669] the Panel considered that additional data are needed. For six substances [FL-no: 02.129, 02.146, 02.147, 02.168, 02.191, and 02.197] information on specifications/stereoisomerism/composition of mixture is missing. For 24 flavouring substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.149, 02.150, 02.171, 02.181, 02.184, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] evaluated using the Procedure the Panel considered that they would present no safety concern at their estimated levels of intake estimated on the basis of the MSDI approach.

KEYWORDS

Flavourings, safety, aliphatic, alicyclic, aromatic, saturated, unsaturated, tertiary alcohols.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) 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, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

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

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

After the completion of the evaluation programme the Union 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, 1996a).

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.18	3 March 2006	http://www.efsa.europa.eu/en/scdocs/scdoc/331.htm	24
FGE.18Rev1	29 January 2008	http://www.efsa.europa.eu/en/scdocs/scdoc/978.htm	30
FGE.18Rev2	30 September 2010		32

The present Revision of FGE.18, FGE.18Rev2, includes the assessment of two additional candidate substances [FL-no: 02.129 and 02.146]. Information on toxicity and/or metabolism on the substance [FL-no: 02.129] is included. No information on toxicity and/or metabolism is available for [FL-no: 02.146]. A search in open literature for did not provide any further data on toxicity or metabolism for these substances.

Since the publication of FGE.18Rev1 additional information toxicity on [FL-no: 02.120, 02.140, 02.144 and 02.197] has become available and is included.

Since the publication of FGE.18Rev1 additional information on specifications on six substances has become available [FL-no: 02.147, 02.168, 02.197, 02.226, 02.230 and 02.253] (EFFA, 2010a).

Based on the additional information made available by Industry, it was also considered appropriate to merge the two subgroups 7 and 8 in the previous revision of this FGE to one subgroup 7 in this current revision of this FGE.

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

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 18, Revision 2

1.1. Description

The present Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 32 saturated and unsaturated aliphatic acyclic and alicyclic tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The 32 flavouring substances under consideration (candidate substances), with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

The present FGE consists of seven aliphatic saturated tertiary alcohols and one ester of such [FL-no: 02.041, 02.052, 02.147, 02.181, 02.184, 02.219, 02.253 and 09.356]; five are aliphatic unsaturated tertiary alcohols which possess isolated terminal double bonds and two are esters thereof [FL-no: 02.123, 02.144, 02.150, 02.168, 02.226, 09.614 and 09.671]; three aliphatic unsaturated tertiary alcohols with conjugated terminal double bonds and one ester of such [FL-no: 02.146, 02.185, 02.191 and 09.669]; one aliphatic unsaturated tertiary alcohol which does not possess terminal double bonds [FL-no: 02.140]; two monocyclic saturated tertiary alcohols and one ester thereof [FL-no: 02.054, 02.171 and 09.617]; two monocyclic unsaturated tertiary alcohols [FL-no: 02.129 and 02.230]; two mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond [FL-no: 02.149 and 02.206]; one bicyclic unsaturated ester [FL-no: 09.808], one tricyclic saturated ester [FL-no: 09.171] and two bi- and tricyclic tertiary alcohols [FL-no: 02.197 and 02.120] and one tertiary alcohol with an aromatic substituent [FL-no: 02.203].

A summary of the safety evaluation is summarised in Table 2a.

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

The 32 candidate substances are closely related structurally to 26 flavouring substances (supporting substances). Twenty-three of these were evaluated in the group of "Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances" and one, menthol, was evaluated in the group "Substances structurally related to menthol" at the 51st JECFA meeting (JECFA, 2000a). Two supporting substances were evaluated at the 63rd JECFA meeting (JECFA, 2005c), one in the group "Aliphatic and alicyclic hydrocarbons" and the other in the group "Aromatic hydrocarbons". The names and structures for the 26 supporting substances are listed in Table 3, together with their evaluation status.

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.).

Nineteen of the 32 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-no: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.191, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For five of these substances [FL-no: 02.146, 02.147, 02.168, 02.191 and 02.197] the stereoisomeric composition has not been specified sufficiently. For four of these flavouring substances [FL-no: 02.147, 02.168, 02.191 and 02.197] 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 (see Table 1).

1.3. Natural Occurrence in Food

Twenty-four out of 32 candidate substances have been reported to occur widely in fruits, liquorice, milk powder, cabbage, mushroom, various herbs, coffee, tea, chicken, wine and rum. Quantitative data on the natural occurrence in food have been reported for 13 of these 32 substances (TNO, 2000).

These reports include among others:

- 2-Methylbutan-2-ol [FL-no: 02.041]: Up to 0.1 mg/kg in cherimoya, up to 0.1 mg/kg in loquat, up to 0.01 mg/kg in passion fruit, 0.00002 mg/kg in sapodilla fruit, 0.0007 mg/kg in chicken, 0.2 mg/kg in rum
- 2-Methylpropan-2-ol [FL-no: 02.052]: 0.25 mg/kg in grape, up to 0.1 mg/kg in mango, 0.0021 mg/kg in guava fruit
- p-Menthane-1,8-diol [FL-no: 02.054]: Up to 0.37 mg/kg in cranberry
- Cedrol [FL-no: 02.120]: 1900 mg/kg in calamus (European), up to 100 mg/kg in cinnamon
- 2-Methylbut-3-en-2-ol [FL-no: 02.123]: 3.3 mg/kg in mango, up to 2.5 mg/kg in coffee, 1 mg/kg in tea, 0.6 mg/kg in black currants, 0.1 mg/kg in cranberry, up to 0.1 mg/kg in passion fruit, 0.05 mg/kg in bilberry, up to 0.05 mg/kg in cherimoya, 0.002 mg/kg in papaya, trace amounts in cabbage, trace amounts in cardamom, 0.003 mg/kg in milk powder
- Elemol [FL-no: 02.149]: 0.37 mg/kg in grapefruit juice
- *p*-Menthan-8-ol [FL-no: 02.171]: 0.01 mg/kg in grapefruit juice
- 2-Methylpentan-2-ol [FL-no: 02.181]: 0.01 mg/kg in plumcot
- 3-Methylpentan-3-ol [FL-no: 02.184]: 0.034 mg/kg in plumcot
- Myrcenol [FL-no: 02.185]: 1.1 mg/kg in liquorice, trace amounts in blueberry, 0.04 mg/kg in grapefruit juice, 0.04 mg/kg in grape
- Ocimenol [FL-no: 02.191]: 0.04 mg/kg in apricot, 0.01 mg/kg in grapefruit juice.

According to TNO eight of the substances have not been reported to occur naturally in any food items. These substances are: 1,2-dihydrolinalool [FL-no: 02.140], 3,6-dimethyloctan-3-ol [FL-no: 02.147], geranyl linalool [FL-no: 02.150], 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197], 2,6-dimethyl-2-heptanol [FL-no: 02.219], 2,4-dimethyl-4-nonanol [FL-no: 02.253], nerolidyl acetate [FL-no: 09.671] and guaiyl acetate [FL-no: 09.808] (TNO, 2000).

2. Specifications

Purity criteria for the 32 substances have been provided by the Flavouring Industry (EFFA, 2004a; EFFA, 2005e; EFFA, 2006k; EFFA, 2007h; EFFA, 2010a; Flavour Industry, 2009c; Flavour Industry, 2009f) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for [FL-no: 02.129 and 02.146] are insufficient as boiling point is missing for [FL-no: 02.129] and for [FL-no: 02.146] is a boiling point and an minimum assay value missing. Furthermore, the stereoisomeric composition / composition of mixture need to be specified for [FL-no: 02.129, 02.146, 02.147, 02.168, 02.191 and 02.197]. Otherwise the specifications are adequate for all 32 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, 1999a).

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, 1999a).

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

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

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

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during

the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present Flavouring Group Evaluation the total annual volume of production of the 32 candidate substances from use as flavouring substances in Europe has been reported to be 10800 kg (EFFA, 2004b; EFFA, 2005e; EFFA, 2006k; EFFA, 2007h; Flavour Industry, 2009c; Flavour Industry, 2009f). For the supporting substances the total annual volume of production is approximately 58000 kg (JECFA, 2000a).

On the basis of the annual volume of production reported for the 32 candidate substances, MSDI values for each of these flavourings have been estimated (Table 2a).

Ninety-four percent of the total annual volume of production for the candidate substances is accounted for by terpineol [FL-no: 02.230] with 10200 kg. The estimated MSDI of terpineol from use as a flavouring substance is 1200 microgram/*capita*/day. The daily *per capita* intakes for each of the remaining substances are less than 30 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 32 candidate substances, information on food categories and normal and maximum use levels[5,6,7] were submitted for 31 of the 32 candidate substances by the Flavour Industry (EFFA, 2004a; EFFA, 2005e; EFFA, 2006k; EFFA, 2007a; EFFA, 2007h; Flavour Industry, 2009c). The 31 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For one of the candidate substances no use levels have been submitted [FL-no: 02.146]. See corresponding entry in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

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

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

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

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

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	31 except [02.197], no data for [FL-no: 02.146]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	31 except [02.197, 02.226], no data for [FL-no: 02.146]
03.0	Edible ices, including sherbet and sorbet	31 except [02.197, 09.671], no data for [FL-no: 02.146]
04.1	Processed fruits	31 except [02.197, 02.226, 02.230], no data for [FL-no: 02.146]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	[09.171], no data for [FL-no: 02.146]
05.0	Confectionery	31 except [02.197], no data for [FL-no: 02.146]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	31 except [02.197, 02.230], no data for [FL-no: 02.146]
07.0	Bakery wares	31 except [02.197], no data for [FL-no: 02.146]
08.0	Meat and meat products, including poultry and game	31 except [02.129, 02.197, 02.226], no data for [FL-no: 02.146]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	31 except [02.129, 02.197, 02.226, 02.230, 02.253], no data for [FL-no: 02.146]
10.0	Eggs and egg products	None, no data for [FL-no: 02.146]
11.0	Sweeteners, including honey	None, no data for [FL-no: 02.146]
12.0	Salts, spices, soups, sauces, salads, protein products etc.	31 except [02.197], no data for [FL-no: 02.146]
13.0	Foodstuffs intended for particular nutritional uses	31 except [02.129, 02.197, 02.226, 02.230], no data for [FL-no: 02.146]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	31 no data for [FL-no: 02.146]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	31 except [02.197], no data for [FL-no: 02.146]
15.0	Ready-to-eat savouries	31 except [02.129, 02.197], no data for [FL-no: 02.146]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	31 except [02.197, 02.226, 02.230], no data for [FL-no: 02.146]

According to the Flavour Industry the normal use levels for the 31 candidate substances for which use level data are available, are in the range of 2 - 200 mg/kg food, and the maximum use levels are in the range of 5 - 500 mg/kg (EFFA, 2002i; EFFA, 2004a; EFFA, 2005e; EFFA, 2006k; EFFA, 2007a; EFFA, 2007h; Flavour Industry, 2009c) (see Table II.1.2, Annex II).

The mTAMDI values for the 20 candidate substances from structural class I (See Section 5) range from 3900 to 14000 microgram/person/day. For the 10 candidate substances from structural class II the mTAMDI values are 3900 microgram/person/day for each of nine candidate substances, and 1600

microgram/person/day for one candidate substance. For the one substance from structural class III the mTAMDI value is 5700 microgram/person/day (see Section 6).

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

Seven of the candidate substances in this group are esters [FL-no: 09.171, 09.356, 09.614, 09.617, 09.669, 09.671 and 09.808]. Hydrolysis data are not available for any of these esters. However, *in vitro* hydrolysis data for the supporting substance linalyl acetate, indicate that these seven esters can be anticipated to be hydrolysed. The carboxylic acids resulting from the hydrolysis of these seven candidate substances are acetic acid, propanoic acid and valeric acid, which will all be incorporated in normal metabolic processes such as beta-oxidation and the citric acid cycle. The alcohols resulting from the hydrolysis of these esters are tertiary alcohols and their metabolisms are considered further below, together with the candidate tertiary alcohols in this Flavouring Group Evaluation.

A consideration of the chemical structures of the candidate substances, their anticipated pathways of metabolism and the extent to which data on one substance may support the metabolism of another substance has indicated that it is appropriate to divide the candidate substances in FGE.18Rev2 into eight subgroups of more closely related structures. This subdivision is shown in Table 4.1.

Table 4.1. Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
1	02.041	2-Methylbutan-2-ol	Aliphatic saturated tertiary alcohols and one ester thereof
	02.052	2-Methylpropan-2-ol	
	02.147	3,6-Dimethyloctan-3-ol	
	02.181	2-Methylpentan-2-ol	
	02.184	3-Methylpentan-3-ol	
	02.219	2,6-Dimethyl-2-heptanol	
	02.253	2,4-Dimethyl-4-nonanol	
	09.356	1,1-Dimethylethyl propionate	
2	02.123	2-Methylbut-3-en-2-ol	Aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two esters thereof
	02.144	2,6-Dimethyloct-7-en-2-ol	
	02.150	Geranyl linalool	
	02.168	Isophytol	
	02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	
	09.614	Linalyl valerate	
	09.671	Nerolidyl acetate	
3	02.146	3,7- Dimethylocta-1,5,7-trien-3-ol	Aliphatic unsaturated tertiary alcohols with conjugated terminal double bonds and one ester thereof
	02.185	Myrcenol	
	02.191	Ocimenol	
	09.669	Myrcenyl acetate	
4	02.140	1,2-Dihydrolinalool	Aliphatic unsaturated tertiary alcohol (without terminal double bond)
5	02.054	p-Menthane-1,8-diol	Monocyclic saturated and unsaturated tertiary alcohols and one ester thereof
	02.129	Bisabola-1,12-dien-8-ol	
	02.171	p-Menthan-8-ol	
	02.230	Terpineol	
	09.617	p-Menthan-8-yl acetate	
6	02.149	Elemol	Monocyclic and bicyclic unsaturated tertiary alcohols with isolated terminal double bonds
	02.206	Sclareol	
7	02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnapthalen-2-ol	Bi- and tricyclic tertiary alcohols and esters
	02.120	Cedrol	
	09.171	Cedryl acetate	

Table 4.1. Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
	09.808	Guaiyl acetate	
8	02.203	2-Phenylpropan-2-ol	Tertiary alcohol with an aromatic substituent

Subgroup 1: Metabolism studies of the three candidate substances 2-methylbutan-2-ol [FL-no: 02.041], 2-methylpropan-2-ol [FL-no: 02.052] and 2-methylpentan-2-ol [FL-no: 02.181] show that these are conjugated with glucuronic acid before excretion in the urine. When rats were given 2-methylpropan-2-ol by gavage, acetone was excreted in small amounts, and when given 2-methylbutan-2-ol by gavage, diols were excreted. This indicates that an additional metabolic pathway of the three candidate substances is oxidation of methyl groups. From these metabolism studies it is anticipated that the candidate substances 2-methylbutan-2-ol [FL-no: 02.041], 2-methylpropan-2-ol [FL-no: 02.052], 3,6-dimethyloctan-3-ol [FL-no: 02.147], 2-methylpentan-2-ol [FL-no: 02.181], 3-methylpentan-3-ol [FL-no: 02.184], 2,6-dimethyl-2-heptanol [FL-no: 02.219], 2,4-dimethyl-4-nonanol [FL-no: 02.253] and the hydrolysis product 2-methylpropan-2-ol from the candidate substance 1,1-dimethylethyl propionate [FL-no: 09.356] are conjugated with glucuronic acid and excreted in the urine, or that they can undergo oxidation to yield the corresponding diols, which are also expected to be excreted as their respective glucuronic acid conjugates.

Subgroup 2: Linalool is a supporting substance to the candidate substances 2-methylbut-3-en-2-ol, linalyl valerate, nerolidyl acetate, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol and geranyl linalool [FL-no: 02.123, 09.614, 09.671, 02.168, 02.226 and 02.150], which all have an isolated terminal double bond in close proximity to the tertiary alcohol group. As these substances or their respective alcohol moieties have a free hydroxyl group, they may be directly conjugated. Seventy-two hours after intragastrical application of 500 mg/kg bw ^{14}C-labelled linalool to 12 weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetrahydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut (Rahman, 1974a). In addition, the metabolism of linalool indicates that these candidate substances may also be metabolised by omega-oxidation of methyl groups and excreted in the urine as the oxidation product as such or after conjugation with glucuronic acid. No oxidation of the terminal double bond in linalool was observed, indicating no formation of epoxide intermediates.

For 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] the structure differs from the supporting substance linalool and the other candidate substances in this group in that the isolated terminal double bond is located distant from the tertiary alcohol group. However, any risk from epoxide formation of this compound is considered to be low since at low dose such epoxides formed are anticipated to be efficiently metabolised by conjugation with glutathione or by epoxide-hydrolase mediated hydrolysis, and in line with the discussion in FGE.07Rev2, the candidate substance 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] can be evaluated via the A-side of the Procedure scheme. Moreover, the tertiary alcohol group can be directly conjugated with glucuronic acid.

Subgroup 3: Myrcene [FL-no: 01.008] is a supporting substance to the four candidate substances 3,7-dimethylocta-1,5,7-trien-3-ol, myrcenol, ocimenol and myrcenyl acetate [FL-no: 02.146, 02.185, 02.191 and 09.669]. These substances also have alcohol moieties that can be directly conjugated. In addition, further oxidation of methyl groups may occur. As shown for myrcene, oxidation of conjugated terminal double bonds in the candidate substances may occur, resulting in epoxide intermediates. However the available genotoxicity data for myrcene do not indicate a genotoxic potential for this substance even in the presence of metabolic activation. Nevertheless, it cannot be anticipated that these candidate substances will be metabolised to innocuous products.

Subgroup 4: 1,2-Dihydrolinalool [FL-no: 02.140] has been shown to be directly conjugated with glucuronic acid like the supporting substance linalool, and excreted. After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep dihydrolinalool and tetrahydrolinalool are formed as metabolites (Rahman, 1974a). *In vivo* metabolism studies in rats on 14C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucoronides (Rahman, 1974a). Additionally, it might be oxidised at the methyl groups, introducing new hydroxyl groups that also can be conjugated and excreted.

Subgroup 5: From the metabolism studies of alpha-terpineol and menthol it is anticipated that the candidate substances terpineol [FL-no: 02.230], p-menthane-1,8-diol [FL-no: 02.054], bisabola-1,12-dien-8-ol [FL-no: 02.129] and p-menthan-8-ol [FL-no: 02.171] (also an hydrolysis product of candidate substance: p-menthan-8-yl acetate [FL-no: 09.617]) may undergo allylic oxidation of the exocyclic methyl group. This could be further oxidised to a carboxylic acid group. Alternative or subsequent metabolism may occur by conjugation with glucuronic acid, followed by excretion in the urine.

Subgroup 6: A metabolism study on elemol [FL-no: 02.149] indicates that the substance is absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of elemol [FL-no: 02.149] and sclareol [FL-no: 02.206], which have the same structural features as elemol, would not be anticipated.

Subgroup 7: Two metabolism studies on cedrol indicate that the candidate substances cedrol [FL-no: 02.120], cedryl acetate [FL-no: 09.171] guaiyl acetate [FL-no: 09.808] and 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197] will be further hydroxylated and excreted in urine as such or as conjugates.

Subgroup 8: In metabolism studies, the supporting substance 1-isopropyl-4-methylbenzene [FL-no: 01.002] (synonym: p-cymene) was oxidised at the isopropyl side chain yielding 2-(p-tolyl)-2-propanol, which is not further oxidised, but excreted unchanged or as a glucuronic acid conjugate. It is anticipated that the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203] will follow the same pathway and be excreted unchanged or in conjugation with glucuronic acid.

In summary, 28 of the candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] are anticipated to be metabolised to innocuous products.

Four of the candidate substances [FL-no: 02.146, 02.185, 02.191 and 09.669] (all in subgroup 3) contain conjugated terminal double bonds and data from a supporting substance, myrcene [FL-no: 01.008] indicate that these may be oxidised, giving rise to epoxide intermediates. Thus, it cannot be anticipated that these three substances will be metabolised to innocuous products. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies and the Procedure can be applied for the safety evaluation of these four candidate substances.

A more detailed discussion of the metabolism of the candidate substances in this evaluation is provided in Annex III.

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

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its

corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the 32 candidate substances from chemical groups 6 and 8 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the 32 substances are summarised in Table 2a.

Step 1

Twenty of the 32 candidate substances of EU chemical groups 6 and 8 are classified into structural class I, 11 candidate substances are classified into stuctural class II and one candidate substance is classified into structural class III according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

Twenty-eigth of the candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] are anticipated to be metabolised to innocuous products and proceed via the A-side of the Procedure scheme.

Four of the candidate substances [FL-no: 02.146, 02.185, 02.191 and 09.669] contain conjugated terminal double bonds and data from the supporting substance myrcene [FL-no: 01.008] indicate that these may be oxidised, giving rise to epoxide intermediates. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies and therefore the Procedure was applied to these four candidate substances [FL-no: 02.146, 02.185, 02.191 and 09.669]. In conclusion, the four substances [FL-no: 02.146, 02.185, 02.191 and 09.669] proceed via the B-side of the Procedure scheme.

Step A3

Seventeen of the candidate substances [FL-no: 02.054, 02.120, 02.140, 02.144, 02.149, 02.168, 02.171, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.614, 09.617, 09.671 and 09.808], which are anticipated to be metabolised to innocuous products have been assigned to structural class I. These substances have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1200 microgram (Table 2a). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

Ten of the candidate substances [FL-no: 02.041, 02.052, 02.123, 02.147, 02.150, 02.181, 02.184, 02.197, 02.203 and 09.356], which are predicted to be metabolised to innocuous products have been assigned to structural class II. These substances have European daily *per capita* intakes (MSDI) of 0.0012 to 12 microgram. These intakes are below the threshold of concern of 540 microgram/person/day for structural class II.

One of the candidate substances [FL-no: 02.129] which is predicted to be metabolised to innocuous products has been assigned to structural class III. This substance has European daily *per capita* intake (MSDI) of 27 microgram. This intake is below the threshold of concern of 90 microgram/person/day for structural class III.

Based on results of the safety evaluation sequence these 28 candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] proceeding via the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances at estimated levels of intake.

Step B3

The four candidate substances (all in subgroup 3), which could not be predicted to be metabolised to innocuous products [FL-no: 02.146, 02.185, 02.191 and 09.669] have been assigned to structural class I and II. These substances have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 12 microgram (Table 2a). These intakes are below the thresholds of concern of 1800 and 540 microgram/person/day for structural class I and II, respectively. Accordingly, they proceed to step B4 of the Procedure.

Step B4

No NOAEL could be derived for any of the four candidate substances proceeding *via* the B-side or for structurally related substances. Accordingly, further data are required for these four candidate substances [FL-no: 02.146, 02.185, 02.191 and 09.669].

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

The estimated intakes for 18 of the 20 candidate substances in structural class I, based on the mTAMDI, are 3900 microgram/person/day, and for the two remaining substances 7000 and 14000 microgram/person/day, respectively. For these 20 candidate substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.

The estimated intakes for nine of the 11 substances assigned to structural class II, based on the mTAMDI, are 3900 microgram/person/day, and for the remaining one substance 1600 microgram/person/day. These are all above the threshold of concern for structural class II substances of 540 microgram/person/day. For one candidate substance [FL-no: 02.146] no use and use levels have been provided by Industry. For comparison of the MSDI and mTAMDI values, see Table 6.1.

The estimated intake for the substance assigned to structural class III, based on the mTAMDI is 5700 microgram/person/day, which is above the threshold of concern for structural class III substances of 90 microgram/person/day. For comparison of the MSDI- and mTAMDI-values see Table 6.1.

Thus, for all the candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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

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

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.054	p-Menthane-1,8-diol	11	3900	Class I	1800
02.120	Cedrol	13	3900	Class I	1800
02.140	1,2-Dihydrolinalool	0.044	3900	Class I	1800
02.144	2,6-Dimethyloct-7-en-2-ol	0.0012	3900	Class I	1800
02.149	Elemol	1.6	3900	Class I	1800
02.168	Isophytol	0.037	3900	Class I	1800
02.171	p-Menthan-8-ol	0.012	3900	Class I	1800
02.206	Sclareol	0.67	3900	Class I	1800
02.219	2,6-Dimethyl-2-heptanol	0.012	3900	Class I	1800
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	0.049	7000	Class I	1800
02.230	Terpineol	1200	14000	Class I	1800
02.253	2,4-Dimethyl-4-Nonanol	0.24	3900	Class I	1800
09.171	Cedryl acetate	0.99	3900	Class I	1800
09.614	Linalyl valerate	0.43	3900	Class I	1800

09.617	p-Menthan-8-yl acetate	0.012	3900	Class I	1800
09.671	Nerolidyl acetate	0.061	3900	Class I	1800
09.808	Guaiyl acetate	0.0012	3900	Class I	1800
02.185	Myrcenol	0.012	3900	Class I	1800
02.191	Ocimenol	0.012	3900	Class I	1800
09.669	Myrcenyl acetate	8.6	3900	Class I	1800
02.041	2-Methylbutan-2-ol	2.7	3900	Class II	540
02.052	2-Methylpropan-2-ol	0.012	3900	Class II	540
02.123	2-Methylbut-3-en-2-ol	0.0012	3900	Class II	540
02.146	3,7-Dimethylocta-1,5,7-trien-3-ol	12		Class II	540
02.147	3,6-Dimethyloctan-3-ol	0.0012	3900	Class II	540
02.150	Geranyl linalool	0.026	3900	Class II	540
02.181	2-Methylpentan-2-ol	0.12	3900	Class II	540
02.184	3-Methylpentan-3-ol	0.0012	3900	Class II	540
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	0.026	1600	Class II	540
02.203	2-Phenylpropan-2-ol	0.0012	3900	Class II	540
09.356	1,1-Dimethylethyl propionate	0.0012	3900	Class II	540
02.129	Bisabola-1,12-dien-8-ol	27	5700	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

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

The four substances for which additional data are requested (See Section 5) [FL-no: 02.146, 02.185, 02.191 and 09.669] and belonging to structural class I and II, will not be included in the combined intake. The combined intakes have been calculated for the remaining candidate and supporting substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2004b), the estimated combined daily *per capita* intake as flavourings of the 17 candidate substances assigned to structural class I is approximately 1200 microgram, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day.

On the basis of the reported annual production volumes in Europe the estimated combined daily *per capita* intake as flavouring of the 10 candidate flavouring substances assigned to structural class II is approximately 3 microgram, which does not exceed the threshold of concern for a compound belonging to structural class II of 540 microgram/person/day.

The 28 candidate substances are structurally related to 26 supporting substances of which 23 were evaluated by JEFCA at its 51st meeting (JECFA, 2000a) and three were evaluated by the JECFA at its 63rd meeting (JECFA, 2005c). The total combined intake (in Europe) of the candidate and the supporting substances all assigned to structural class I is approximately 24000 microgram/*capita*/day, which exceeds the threshold of concern for the corresponding structural class (1800 microgram/person/day).

However, the major contribution to the total combined intake of flavouring substances assigned to structural class I (94 %) is provided by four supporting substances, namely menthol [FL-no: 02.015] (16000 microg/*capita*/day), linalool [FL-no: 02.013] (2200 microg/*capita*/day), alpha-terpineol [FL-no: 02.014] (2600 microg/*capita*/day) and linalyl acetate [FL-no: 09.013] (1700 microg/*capita*/day).

The estimated intake of menthol [FL-no: 02.015] and of alpha-terpineol [FL-no: 02.014] corresponds to 0.310 mg/kg bw/day. This represents 8 % of the acceptable daily intake (ADI) of 4 mg/kg bw/day for menthol established at the 51st JECFA meeting (JECFA, 2000a).

The reported NOAEL of 24 mg/kg bw/day for linalyl acetate (the lowest reported NOAEL) is 340-fold higher than the estimated combined intake of 0.07 mg/kg bw/day of linalool [FL-no: 02.013] and linalyl acetate [FL-no: 09.013].

Excluding the four major contributors (menthol, linalool, alpha-terpineol and linalyl acetate), the total estimated combined intake (in Europe) for the candidate and supporting substances belonging to structural class I is approximately 552 microgram/*capita*/day, which does not exceed the threshold of concern for the corresponding structural class (1800 microgram/person/day).

The total estimated combined intake (in Europe) of the candidate and the supporting substances assigned to structural class II is 6.5 microgram/*capita*/day, which does not exceed the threshold of concern for the corresponding structural class (540 microgram/person/day).

The intake of the one candidate substance assigned to structural class III is 27 microgram/*capita*/day. No supporting substances are assigned to structural class III and thus calculation of a combined intake for this substance is not applicable.

8. Toxicity

8.1. Acute Toxicity

Data are available for 16 of the 32 candidate substances and for 19 of the 26 supporting substances. The oral LD_{50} values in rats, mice or rabbits ranged from 230 to 50000 mg/kg body weight (bw).

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

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data are available for six of the candidate substances, 2-methylpropan-2-ol [FL-no: 02.052], cedrol [FL-no: 02.120], 2-methylbut-3-en-2-ol [FL-no: 02.123], bisabola-1,12-dien-8-ol [FL-no: 02.129], 2,6-Dimethyloct-7-en-2-ol [FL-no: 02.144], and sclareol [FL-no: 02.206] (see Annex IV, Table IV.2).

A number of studies have been conducted on 2-methyl-propan-2-ol [FL-no: 02.052], as described below:

In a 10-week study in male rats, exposure to drinking water containing 0.5 % 2-methylpropan-2-ol [FL-no: 02.052] (equivalent to 500 mg/kg bw/day) resulted in histopathological changes in the liver and kidney. No other tissues were examined or analyses performed (Acharya et al., 1997).

Ninety-day studies were performed in Fisher F344/N rats and $B6C3F_1$ mice, in conjunction with 2 year chronic toxicity/carcinogenicity studies, which are described below. The main findings after 90 days were, in rats, hyperplasia and inflammation of the urinary bladder in males receiving 2 % and males and females receiving 4 % 2-methylpropan-2-ol in the drinking water (equivalent to 2000 and 4000 mg/kg bw/day, respectively), increased incidence of nephropathy in females receiving 0.5 %, 1 % and 4 % (equivalent to 500, 1000 and 4000 mg/kg bw/day) and increased severity of nephropathy in males at all treatment doses (intakes of 250 mg/kg bw/day and above). In mice, transitional epithelial hyperplasia and inflammation were observed in the urinary bladder in 2 % and 4 % group males (equivalent to 5000 and 10,000 mg/kg bw/day, respectively) and 4 % group females (equivalent to

10,000 mg/kg bw/day) (Lindamood et al., 1992; NTP, 1995b). These observations were consistent with earlier 90-day studies using the same species and strains and the same dose levels, except that there was no reference in the short report to renal effects in rats (Brown & Wheeler, 1979).

In the 2-year study on 2-methylpropan-2-ol [FL-no: 02.052] in F344/N rats, the animals (60/sex/group) were dosed via drinking water containing, for males 0, 0.125, 0.25 or 0.5 % (equivalent to 0, 90, 200 or 420 mg/kg bw/day) and for females 0, 0.25, 0.5 or 1 % (equivalent to 0, 180, 330 or 650 mg/kg bw/day), 2-methylpropan-2-ol. Survival was significantly reduced in the 0.5 % male group and 1 % female group. Severity of nephropathy and incidence and severity of transitional cell nephropathy were reported to be increased in all treated groups. Foci of mineralisation were observed in the renal papillas in all treated groups, and the incidence of renal mineralisation was significantly increased in the 0.5 % male group. The incidences of focal renal tubular hyperplasia and adenoma were observed to be increased in a dose-related manner in all treated male groups but did not reach statistical significance. Combined incidences of renal tubular adenomas and carcinomas were significantly increased in the 0.25 % male group, although not in the 0.5 % dose group. Renal tubular hyperplasia was observed in one female in the 1 % dose group (NTP, 1995b). A review subcommittee for the US National Toxicology Program (NTP) concluded that the study showed 'some evidence of carcinogenic activity' in the males and 'no evidence of carcinogenic activity' in the females (NTP, 1995b).

Further examination of renal samples from the 90-day study in F344/N male rats revealed a significant increase in the quantity of hyaline droplets and number of intracytoplasmic deposits of abnormal shape (crystalline rhomboid structures) in treated groups compared to controls, indicating that the nephropathy observed in male rats was at least in part due to alpha-2 μ-globulin (Takahashi et al., 1993). The NTP review subcommittee noted that the increased severity of nephropathy also seen in females indicated that the mechanism for renal toxicity is not limited to increased accumulation of alpha-2 μ-globulin (NTP, 1995b) ; however, tumours were observed in males only and appear to be related to a dose-related increase in renal tubule hyperplasia, which occurred in males only.

The $B6C3F_1$ mice (60/sex/group) received drinking water containing 0, 0.5, 1 or 2 % 2-methylpropan-2-ol [FL-no: 02.052] (equivalent to 0, 540, 1040, 2070 mg/kg bw/day in males and 0, 510, 1020 or 2100 mg/kg bw/day in females) for 2 years. Survival of the 2 % male group was significantly reduced compared to controls. Incidence of follicular cell hyperplasia of the thyroid were significantly increased in all treated male groups and in the 1 % and 2 % female groups. Incidence of follicular cell adenoma was significantly increased in the 2 % female group. The combined incidences of follicular cell adenomas and carcinomas was observed to be increased in the 1 % male group, although this did not reach statistical significance. However, the incidence of adenoma exceeded the highest incidence seen in historic NTP drinking water controls. Chronic inflammation of the urinary bladder was significantly increased in the 2 % male and female groups, and transitional cell hyperplasia in the urinary bladder was significantly increased in the 2 % female group. No NOAEL could be derived from this study for males; the NOAEL in females was 0.5 % in the diet, equivalent to 510 mg/kg bw/day (NTP, 1995b). An NTP review subcommittee concluded that the study demonstrated 'equivocal evidence of carcinogenic activity' in male mice and 'some evidence of carcinogenic activity' in females (NTP, 1995b). The Panel concluded that these tumours appear to be secondary to follicular cell hyperplasia, which was observed in all treated male groups and mid- and high-dose female groups.

The Panel concluded, taking into account the clear lack of a genotoxic potential *in vivo*, that the carcinogenic effects observed in male rats and in mice appear likely to be due to threshold-based mechanisms. Therefore, the overall results do not preclude evaluating 2-methylpropan-2-ol and structurally related candidate substances through the Procedure.

In a 90-day study, 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] was administered by gavage to ten male and ten female Sprague-Dawley rats at dose levels of 10, 50, 500 and 1000 mg/kg bw/day. A control group of ten males and ten females was dosed with vehicle (corn oil) alone (Dunster et al. 2006). In

female rats, treatment related effects such as decrease in body weight, platelet counts, thromboplastin time, haemoglobin, hematocrit, erythrocyte counts, increase in serum aspartate aminotransferase, alanine aminotransferase, urinary creatinine were observed doses of 500 and/or 1000 mg/kg bw/day. No such changes were observed in females at the lower doses tested and therefore, 50 mg/kg bw/day was considered to constitute a No Observed Adverse Effect Level (NOAEL) for females. In the kidney of male rats, a greater incidence and/or severity of groups of basophilic tubules and/or globular accumulations of eosinophilic material were observed at all doses tested. Dunster et al., 2007 considered these findings to be consistent with the presence of hydrocarbon nephropathy, which results from the excessive accumulation of alpha2-urinaryglobulin. The latter was indicated by positive staining with Mallory' s Heidenhain stain in renal proximal tubular epithelial cells, frequently associated with tubular degenerative changes. alpha2-Urinaryglobulin is found only in the proximal tubular epithelium of adult male rats. Dunster et al. 2007, concluded that this effect is not indicative of a hazard to human health and that 10 mg/kg/day should be regarded as the NOAEL for males (Dunster et al., 2007).

A NOAEL of 150 mg/kg bw/day in rats is available from a 28-day oral toxicity study conducted on 2-methylbut-3-en-2-ol [FL-no: 02.123] (BASF, 1994a).

A NOAEL of 850 mg/kg bw/day in rats is available from a 28-day oral toxicity study conducted on bisabola-1,12-dien-8-ol [FL-no: 02.129] (Habersang et al., 1979).

A 32-day oral toxicity study on both sclareol [FL-no: 02.206] and cedrol [FL-no: 02.120] was conducted in Charles River Laboratories CD (SD) rats. Two groups of 10 rats received dose levels of approximately 8.8 mg/kg bw/day of sclareol and two groups of 10 rats received dose levels of approximately 8.4 mg/kg bw/day of cedrol. The animals were dosed seven days per week via gavage. No adverse effects were observed (IOFI, 2006a).

Data are available for six supporting substances [FL-no: 01.008, 02.013, 02.015, 09.013, 09.423 and 09.830] and two other related substances (linalyl cinnamate and geranyl acetate) administered as a mixture together with citronellyl acetate (See Annex IV, Table IV.1).

Linalool [FL-no: 02.013] was reported to result in no significant adverse effects compared to control when administered to rats at 50 mg/kg bw/day as a 50 % mixture with citronellol via the diet for 84 days. No further details are available. Similarly linalyl acetate [FL-no: 09.013], linalyl isobutyrate [FL-no: 09.423] and geranyl acetate [FL-no: 09.011] administered as a mixture at doses of dosed 24, 27 and 48 mg/kg bw/day respectively resulted in no adverse effects (Oser, 1967).

No adverse effects were reported in rats (10/sex/group) given diets containing 0, 1000, 2500 or 10,000 ppm linalyl isobutyrate [FL-no: 09.423] (equivalent to 0, 50, 125 or 500 mg/kg bw/day) (Hagan et al., 1967) for 18 weeks. Few study details are available. Similarly no adverse effects were reported in a similar study on terpinyl acetate [FL-no: 09.830] given diet containing 0, 1000, 2500 or 10,000 ppm (equivalent to 0, 50, 125 or 500 mg/kg bw/day) for 20 weeks (Hagan et al., 1967).

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

8.3. Developmental / Reproductive Toxicity Studies

Data are available for two candidate substances: 2-methylpropan-2-ol [FL-no: 02.052] and 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144].

In a study on 2-methylpropan-2-ol [FL-no: 02.052], doses of 0.5, 0.75 or 1 % liquid diet (equivalent to approximately 3400, 4900 and 6400 mg/kg bw/day) administered to mice on days 6-20 of gestation were associated with reduced performance of offspring in four neurobehavioural tests: righting reflex, cliff avoidance and open field (conducted on days 2, 4, 6, 8 and 10 post-parturition) and roto-rod (conducted on days 14, 16, 18, 20 and 22). The authors indicated that there was some evidence of

recovery in the first three tests but not the latter during the periods of study (Daniel & Evans, 1982). In a second study, administration of 1557 mg/kg bw/day to pregnant CBA/J and C57BL/6J mice by oral gavage during days 6-18 of gestation resulted in a significant increase in the incidence of foetal resorptions and a significant decrease in the number of live births per litter. No foetal malformations were observed (Faulkner et al., 1989). The Panel noted that these experimental studies were conducted using very high doses and were not of concern when compared with the low exposure arising from use as flavouring substances.

In a study on 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], doses of 0, 250, 500 or 1000 mg/kg bw/day were administered to Charles River Laboratory CD(SD) rats via corn oil gavage on gestational days 7-17. Observations for viability, adverse clinical signs, abortion, and premature delivery were conducted before and approximately one hour following treatment and once thereafter. Body weight gains in the high-dose group were reduced by 5 % when compared to controls; weight losses were observed after the first two doses. Although these observations were not significant, they were considered to be evidence of a threshold level for maternal toxicity. Both maternal absolute and relative feed consumption values were significantly reduced in the 1000 mg/kg bw/day group compared to vehicle control. Reduced feed consumption was most prominent on gestational days 7-10, which correlated with the weight losses and reduced weight gains that occurred during the initial days of the dosing period. Body weights for combined male and female foetuses were reduced approximately 3 % in the 1000 mg/kg bw per day group compared to vehicle controls (the reduction was statistically significant for females). No other litter parameters were affected by dosages of 2,6-dimethyloct-7-en-2-ol as high as 1000 mg/kg bw per day. Upon inspection, there were no foetal gross external alterations or foetal soft tissue or skeletal malformations observed in the experiment. There were no observable soft tissue variations, and skeletal variations were limited to two reversible minor changes. First, there was evidence of a threshold (but statistically significant) increase in supernumerary ribs, along with associated significant increases and decreases in the respective numbers of thoracic and lumbar vertebrae. Second, there was evidence of a small but statistically significant retardation in ossification of the metatarsal bones in the hind paws, evident as a reduction in the mean number of ossified metatarsal bones. The results indicate that 1000 mg 2,6-dimethyloct-7-en-2-ol/kg bw per day produced threshold levels of maternal and developmental toxicity. As such, the maternal and developmental no-observable-adverse-effect levels (NOAELs) for 2,6-dimethyloct-7-en-2-ol are considered to be 500 mg/kg/day (Politano et al., 2008).

Data are available for three supporting substances [FL-no: 02.013, 02.015 and 01.008] (See Annex IV, Table IV.3).

A NOAEL of 365 mg/kg bw/day was reported for linalool [FL-no: 02.013]. A higher dose of 729 mg/kg bw/day resulted in decreased live litter size and increased pup mortality; maternal toxicity was observed at all doses tested (Hoberman & Christian, 1989). NOAELs of 185 to 425 mg/kg bw/day (highest doses tested) were reported for menthol [FL-no: 02.015] in teratological studies in rats, mice, hamsters and rabbits (Food and Drug Research Laboratories, Inc., 1973). Three studies have been performed in rats on myrcene [FL-no: 01.008]; two developmental toxicity studies (one in which myrcene was administered to dams on days 6-15 of gestation and one in which dosing was from day 15 of gestation until weaning) and a single generation study. NOAELs were 500 mg/kg bw/day, 250 mg/kg bw/day and 300 mg/kg bw/day, respectively (Delgado et al., 1993a; Delgado et al., 1993b; Paumgartten et al., 1998).

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

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for nine candidate [FL-no: 02.052, 02.041, 02.120, 02.123, 02.129, 02.140, 02.144, 02.168 and 02.197] and for eight supporting substances [FL-no: 01.002, 01.008, 02.013, 02.014, 02.015, 02.097, 09.013 and 09.830]. Data from *in vivo* tests are available for

two candidate [FL-no: 02.052 and 02.123] and for three supporting substances [FL-no: 01.008, 02.013 and 02.015].

2-Methylpropan-2-ol [FL-no: 02.052] was negative in reversion tests in *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100, without and with metabolic activation by rat and hamster liver S9 (Zeiger et al., 1987). A borderline (less than two-fold) increase in revertants in strain TA1535 was observed in two other studies (Haworth et al., 1981a; Haworth et al., 1981b), which were not available for evaluation. A marginal increase in sister chromatid exchange (SCE) was reported from two studies with Chinese hamster ovary (CHO) cells, which could not be evaluated because the papers were submitted incompletely (Putman, 1985; Thilagar et al., 1981). A borderline increase in mutant frequency was observed in mouse-lymphoma TK +/- cells in a single test in the absence of metabolic activation, whereas negative results were obtained in repeated experiments with S9 (McGregor et al., 1988b). Again, similar results are quoted in the summary of an unpublished study not available for evaluation (Kirby et al., 1981). Finally, a slight increase of petite (mitochondrial) mutations was reported in yeast after treatment with 2-methylpropan-2-ol (Jiménez et al., 1988), but this effect is not considered relevant for genotoxicity assessment.

1,2-Dihydrolinalool [FL-no: 02.140] was negative in *S. typhimurium* TA97, TA100, TA102, TA1535, without and with metabolic activation (Gocke, 1999a). In one test, 1,2-dihydrolinalool was negative without S9 but increased the number of revertants in TA98 with metabolic activation. This positive finding could not be reproduced in subsequent tests with TA98 (Gocke, 1999a).

The remaining candidate substances, for which genotoxicity data have become available in this Revision 2 of FGE.18; Bisabola-1,12-dien-8-ol [FL-no: 02.129], cedrol [FL-no: 02.120], 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197] were all negative in reversion tests in the following test objects, without and with metabolic activation by rat liver S9: [FL-no: 02.129]: *S. typhimurium* TA97a, TA98, TA100, TA102, TA1535, TA1537 up to 5000 µg/plate; [FL-no: 02.120]: *S. typhimurium* TA97a, TA98, TA102, TA1535 up to 5000 µg/plate; [FL-no: 02.144]: *S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 up to 5000 µg/plate; and [FL-no: 02.197]: *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA up to 250 µg/plate without metabolic activation and up to 500 µg/plate with metabolic activation. The studies were considered valid.

A negative result was obtained in a sister chromatid exchange (SCE) study on bisabola-1,12-dien-8-ol [FL-no: 02.129] with Chinese hamster ovary (CHO) cells. The data were presented in a review, but considered valid.

In vivo, 2-methylpropan-2-ol gave clearly negative results in a rat bone marrow micronucleus test, after intraperitoneal (i.p.) administration of a range of doses (six doses from 39 to 1250 mg/kg bw), which reached complete lethality at the highest dose (NTP, 1997c). Negative results were also obtained in the mouse peripheral blood micronucleus assay, after 13 weeks of oral exposure to 3000 to 40000 ppm in drinking water. There was no deviation in the PCE/NCE ratio in treated animals, but signs of general toxicity were observed at the two highest doses, indicating significant systemic exposure (NTP, 1995b). In another study, 2-methylpropan-2-ol was negative in the mouse bone marrow micronucleus assay when given by ip injections at doses up to 1250 mg/kg bw (three daily administrations) (NTP, 1996c). The alleged positive result obtained with 2-methylpropan-2-ol in a rat bone marrow chromosomal aberration test after oral administration of 1/5 of the LD_{50} (Barilyak & Kozachuk, 1988) is considered inconclusive, because the result is not adequately supported by experimental data.

Terpineol [FL-no: 02.230] (the mixture of alpha-terpineol, beta-terpineol, delta-terpineol and gamma-terpineol) was tested negative in a rec assay in *Bacillus subtilis* (Oda et al., 1978).

Alpha-terpineol [FL-no: 02.014] was reported to give weakly positive results (as a dose-dependent increase in mutation frequency both with and without S9 activation with a maximum increase of 2.2-

fold compared with the control) in an Ames-type mutagenicity assay in one (TA102) of four *S. typhimurium* strains tested (TA97a, TA98, TA100, TA102). alpha-Terpineol was incorporated into agar plates up to 2500 microgram/plate, either with or without S9 metabolic activation (Gomes-Carneiro et al., 1998).

In other studies, alpha-terpineol gave consistently negative results in Ames assays in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, either with or without S9 metabolic activation (Florin et al., 1980; Lorillard, 1983b; Heck et al., 1989).

In an *in vivo/in vitro* study designed to investigate the mutagenicity of the metabolites of beta-terpineol [FL-no: 02.097], Sprague-Dawley rats were administered a single dose of 0.5 ml (452 mg) of beta-terpineol by gavage and the urine was collected for 24-hours. The urine (500 microl) was hydrolysed with beta-glucuronidase. Hydrolysed and un-hydrolysed urine samples, ether extracts of the urine, and aqueous fractions of the urine-ether extracts were then separately incubated with *S. typhimurium* strains TA98 and TA100 without S9 activation. Neither beta-terpineol, nor any of the urinary solutions isolated from the urine of rats given 452 mg doses of beta-terpineol, showed any evidence of mutagenicity in either TA98 or TAl00 without metabolic activation (Rockwell & Raw, 1979).

In gene mutation tests in mouse lymphoma cells, alpha-terpinol was non-mutagenic when applied of doses up to 250 nl/ml (with S9) and 300 nl/ml (without S9) (Lorillard, 1982); negative results were also obtained in another study in which alpha-terpineol was tested up to 0.5 µl/ml (without S9) and 0.75 µl/ml (with S9) (Kirby et al., 1984). Based on the negative results obtained in gene mutation tests in mammalian cells, and in view of the sensibility of the TK+ /- system to mutagens specifically active toward the *S. typhimurium* strain TA102, the Panel concluded that alpha-terpineol does not raise concern for genotoxicity.

Overall, 2-methylpropan-2-ol provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence does not raise concern for *in vivo* genotoxicity.

2-Methylbut-3-en-2-ol [FL-no: 02.123] was reported to be negative in two bacterial gene mutation tests and in an *in vivo* micronucleus test; however, the unpublished study reports are not available for re-evaluation.

The available data considered valid do not give rise to safety concerns with respect to genotoxicity.

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

9. Conclusions

In the present Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2) seven of the candidate substances are aliphatic saturated tertiary alcohols and one is an ester of such, five are aliphatic unsaturated tertiary alcohols which possess isolated terminal double bonds and two are esters of such, three are aliphatic unsaturated tertiary alcohols with conjugated terminal double bonds and one is an ester of such, one is an aliphatic unsaturated tertiary alcohol which does not possess terminal double bonds, two are monocyclic saturated tertiary alcohols and one is an ester of such, two are a monocyclic unsaturated tertiary alcohol, two are mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond, one is a bicyclic unsaturated ester, three are bi- and tricyclic tertiary alcohols and one is a tertiary alcohol with an aromatic substituent.

Nineteen of the 32 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-no: 02.120, 02.129, 02.140,

02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.191, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For five of these substances [FL-no: 02.146, 02.147, 02.168, 02.191 and 02.197] the stereoisomeric composition has not been specified sufficiently. For four of these five substances [FL-no: 02.147, 02.168, 02.191 and 02.197] the stereoisomeric composition of the mixture has not been specified.

Twenty of the 32 candidate substances are classified into structural class I, 11 candidate substances are classified into stuctural class II and one is classified into structural class III according to the decision tree approach.

Twenty-four out of the 32 candidate substances have been reported to occur in a wide range of food items.

According to the default MSDI approach, 31 of the 32 flavouring substances in this group have intakes in Europe from 0.0012 to 27 microgram/*capita*/day, which are below the thresholds of concern for structural class I (1800 microgram/person/day), structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day). The remaining substance, terpineol [FL-no: 02.230] belonging to structural class I, has an MSDI of 1200 microgram/*capita*/day, which is also below the threshold for this structural class.

On the basis of the reported annual production volumes in Europe, the combined intakes of 17 candidate substances belonging to class I and of 10 candidate substances belonging to class II would result in combined intakes of approximately 1200 and 3 microgram/*capita*/day, respectively. These values are lower than the thresholds of concern for structural class I and class II substances of 1800 and 540 microgram/person/day, respectively. (The four substances for which additional data are requested are not included in the combined intake). The total combined intake of the candidate and supporting substances belonging to structural class I is approximately 24000 microgram/*capita*/day. This total combined intake exceeds the threshold for structural class I of 1800 micrograms/person/day. However, based on the available toxicological data and the anticipated efficient metabolism this combined intake is not considered to be of safety concern. The total combined intake of the candidate and supporting substances belonging to structural class II is approximately 6.5 microgram/*capita*/day, which is below the threshold of concern for structural class II (540 microgram/person/day).

2-Methylpropan-2-ol [FL-no: 02.052] provided an equivocal evidence of genotoxicity in some *in vitro* assays, while it was clearly negative *in vivo* in cytogenetic tests conducted up to the maximum tolerated dose. The overall weight of the experimental evidence and the lack of structural alerts for genotoxicity for this substance and its metabolites do not raise concern for *in vivo* genotoxicity. For the other substances in this group the available data considered valid do not give rise to any safety concerns with respect to genotoxicity.

Twenty-eight of the candidate substances are anticipated to be metabolised to innocuous products. For the remaining four candidate substances no metabolism data are available and therefore they cannot be predicted to be metabolised to innocuous products. No appropriate NOAEL was available for these four candidate substances [FL-no: 02.146, 02.185, 02.191 and 09.669] or for the supporting substances. Therefore, additional data are required for these four candidate substances.

It is considered that, on the basis of the default MSDI approach, 28 of the 32 candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. For the remaining four substances [FL-no: 02.146, 02.185, 02.191 and 09.669], no appropriate NOAEL was available or for the supporting substances. Therefore, additional data are required for these four candidate substances.

The mTAMDI values for the 20 candidate substances from structural class I range from 3900 to 14000 microgram/person/day. For 10 of the 11 candidate substances from structural class II, the mTAMDI values are 3900 microgram/person/day for each of nine candidate substances, and 1600 microgram/person/day for one candidate substance. For one candidate substance [FL-no: 02.146] from structural class II no data on use and use levels are provided. For the one candidate substance from structural class III the mTAMDI is 5700 microgram/person/day. Accordingly, the estimated intakes for the 31 candidate substances for which use levels are provided are above the thresholds of concern for their structural classes. Therefore, for these 31 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 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for all 32 candidate substances. However, information on the stereoisomeric composition has not been specified sufficiently for five substances [FL-no: 02.146, 02.147, 02.168, 02.191 and 02.197]. In addition, the composition of the mixture of [FL-no: 02.129] is missing and for [FL-no: 02.129 and 02.146] are other information on specifications lacking. Thus, the final evaluation of the materials of commerce cannot be performed for six substances [FL-no: 02.129, 02.146, 02.147, 02.168, 02.191 and 02.197], pending further information.

In conclusion, for four flavouring substances [FL-no: 02.146, 02.185, 02.191 and 09.669] the Panel considered that additional data are needed. For six substances [FL-no: 02.129, 02.146, 02.147, 02.168, 02.191 and 02.197] information on specifications/stereoisomerism/composition of mixture is missing. For 24 flavouring substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.149, 02.150, 02.171, 02.181, 02.184, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.171, 09.356, 09.614, 09.617, 09.671 and 09.808] evaluated using the Procedure the Panel considered that they would present no safety concern at their estimated levels of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 18, REVISION 2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation18, Revision 2

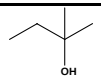
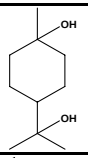
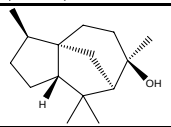
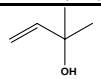
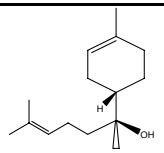
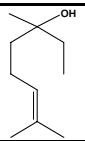
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.041	2-Methylbutan-2-ol		515 75-85-4	Liquid $C_5H_{12}O$ 88.15	Slightly soluble 1 ml in 1 ml	102 MS 96 %	1.402-1.408 0.805-0.813	
02.052	2-Methylpropan-2-ol		698 75-65-0	Liquid $C_4H_{10}O$ 74.12	Slightly soluble 1 ml in 1 ml	82 MS 95 %	1.384-1.390 0.780-0.790	
02.054	p-Menthane-1,8-diol		701 80-53-5	Solid $C_{10}H_{20}O_2$ 172.27	Slightly soluble 1 ml in 1 ml	260 116 MS 95 %	n.a. n.a.	
02.120	Cedrol		10190 77-53-2	Solid $C_{15}H_{26}O$ 222.37	Practically insoluble or insoluble 1 ml in 1 ml	286 86 MS 95 %	n.a. n.a.	
02.123	2-Methylbut-3-en-2-ol		11794 115-18-4	Liquid $C_5H_{10}O$ 86.13	Practically insoluble or insoluble 1 ml in 1 ml	97 MS 98 %	1.413-1.419 0.818-0.827	
02.129	Bisabola-1,12-dien-8-ol	 (l)-alpha-Bisabolol shown	4666 10178 23089-26-1	Liquid $C_{15}H_{26}O$ 222.37	Insoluble Soluble	 MS >94 %	1.493-1.499 0.927-0.935	BP 8). Register name to be changed to (l)-alpha-Bisabolol. Secondary components (no amount given): (d)-alpha-Bisabolol (CAS No. 23178-88-3), (l)-epi-alpha-Bisabolol (CAS No.78148-59-1), (d)-epi-alpha-Bisabolol (CAS No. 76738-75-5).
02.140	1,2-Dihydrolinalool		2270-57-7	Liquid $C_{10}H_{20}O$ 156.27	Practically insoluble or insoluble 1 ml in 1 ml	90 (16 hPa) MS 95 %	1.452-1.458 0.857-0.863	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation18, Revision 2

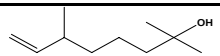
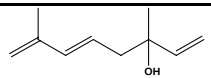
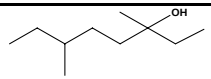
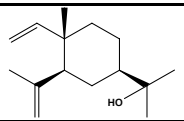
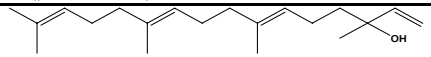
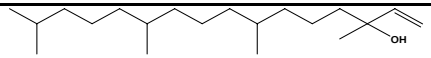
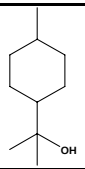
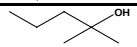
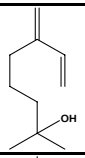
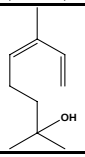
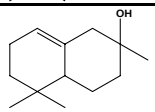
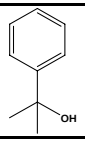
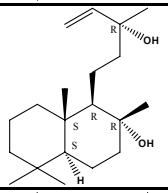
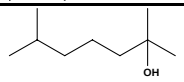
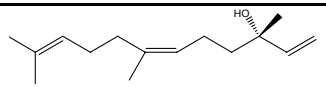
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.144	2,6-Dimethyloct-7-en-2-ol		 18479-58-8	Liquid $C_{10}H_{20}O$ 156.27	Practically insoluble or insoluble 1 ml in 1 ml	86 (15 hPa) MS 95 %	1.434-1.440 0.824-0.830	Racemate.
02.146	3,7-Dimethylocta-1,5,7-trien-3-ol 6)		10202 29957-43-5	Liquid	Insoluble	 MS	1.489-1.495 0.878-0.886	AV 7), BP 8). CASrn in Register to be changed to 53834-70-1, which covers (E)-3,7-Dimethylocta-1,5,7-trien-3-ol. Register name to be changed to (E)-3,7-Dimethylocta-1,5,7-trien-3-ol. (R)- or (S)-enantiomer not specified by CASrn 53834-70-1.
02.147	3,6-Dimethyloctan-3-ol		 151-19-9	Liquid $C_{10}H_{22}O$ 158.28	Practically insoluble or insoluble 1 ml in 1 ml	195 MS 95 %	1.434-1.440 0.831-0.837	Mixture of diastereo isomers (EFFA, 2010a). Stereoisomeric composition to be specified.
02.149	Elemol		10205 639-99-6	Solid $C_{15}H_{26}O$ 222.37	Practically insoluble or insoluble 1 ml in 1 ml	123 (5 hPa) 50 MS 95 %	n.a. n.a.	Register name to be changed to (-)-alpha-elemol = (1R,3S,4S)-isomer.
02.150	Geranyl linalool		 1113-21-9	Solid $C_{20}H_{34}O$ 290.49	Practically insoluble or insoluble 1 ml in 1 ml	153 (5 hPa) 51 MS 95 %	1.484-1.490 0.878-0.881	Racemate of (6E,10E)-isomer. Name to be changed accordingly.
02.168	Isophytol		10233 505-32-8	Liquid $C_{20}H_{40}O$ 296.54	Practically insoluble or insoluble 1 ml in 1 ml	138 (0.1 hPa) MS 95 %	1.457-1.462 0.847-0.853	Mixture of diastereo isomers (EFFA, 2010a). Stereoisomeric composition to be specified.
02.171	p-Menthan-8-ol		 498-81-7	Solid $C_{10}H_{20}O$ 156.27	Practically insoluble or insoluble 1 ml in 1 ml	208 35 MS 95 %	1.460-1.466 0.909-0.915	
02.181	2-Methylpentan-2-ol		 10274 590-36-3	Liquid $C_6H_{14}O$ 102.18	Slightly soluble 1 ml in 1 ml	122 MS	1.409-1.415 0.823-0.829	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation18, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
02.184	3-Methylpentan-3-ol		10277 77-74-7	Liquid $C_6H_{14}O$ 102.18	Practically insoluble or insoluble 1 ml in 1 ml	121 MS 95 %	1.415-1.421 0.824-0.830	
02.185	Myrcenol		543-39-5	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble 1 ml in 1 ml	91 (13 hPa) MS 95 %	1.470-1.476 0.873-0.879	
02.191	Ocimenol		5986-38-9	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble 1 ml in 1 ml	94 (16 hPa) MS 95 %	1.480-1.486 0.871-0.877	Mixture of (E)- and (Z)-isomers (EFFA, 2010a). Composition of stereoisomeric mixture to be specified.
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol		10173 41199-19-3	Solid $C_{13}H_{22}O$ 194.32	Practically insoluble or insoluble 1 ml in 1 ml	82 (1 hPa) 68 MS 95 %	n.a. n.a.	Mixture of diastereo isomers (EFFA, 2010a). Stereoisomeric composition to be specified.
02.203	2-Phenylpropan-2-ol		11704 617-94-7	Liquid $C_9H_{12}O$ 136.19	Practically insoluble or insoluble 1 ml in 1 ml	218 MS 95 %	1.529-1.535 0.971-0.977	
02.206	Sclareol		10311 515-03-7	Solid $C_{20}H_{36}O_2$ 308.5	Practically insoluble or insoluble 1 ml in 1 ml	182 (1.33 hPa) 106 MS 98 %	n.a. n.a.	
02.219	2,6-Dimethyl-2-heptanol		13254-34-7	Liquid $C_9H_{20}O$ 144.26	Practically insoluble or insoluble 1 ml in 1 ml	171 MS 98 %	1.421-1.427 0.816-0.822	
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol		67 142-50-7	Liquid $C_{15}H_{26}O$ 222.37	Practically insoluble or insoluble Soluble	291.92 20.98 MS 95 %	1.478-1.483 0.870-0.876	Register name to be changed to [S-(cis)]-3,7,11-Trimethyl-1,6,10-

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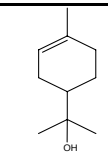
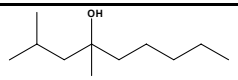
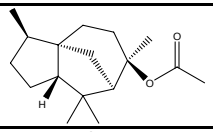
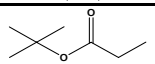
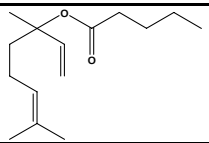
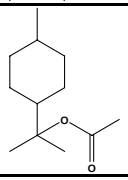
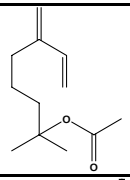
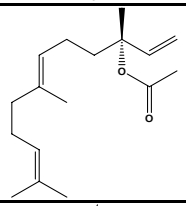
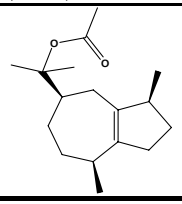
								dodecatrien-3-ol (Also called S-(Z)-(+)-nerolidol (EFFA, 2010a).
02.230	Terpineol	 alfa-Terpineol shown	8000-41-7	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	210-24 to 235-6 MS 91-99 %	1.480-1.488 0.928-0.937	The specification covers alpha-, beta-, gamma- and delta-terpineol. Composition of mixture: 55-75 % alpha, 16-23 % gamma, 1-10 % cis-beta, 1-13 % trans-beta, 0-1 % delta (EFFA, 2007h).
02.253 1850	2,4-Dimethyl-4-Nonanol		4407 74356-31-3	Liquid $C_{11}H_{24}O$ 172.31	Practically insoluble or insoluble 1 ml in 1 ml	212 MS 95 %	1.434-1.440 0.825-0.833	Racemate (EFFA, 2010a).
09.171	Cedryl acetate		527 77-54-3	Solid $C_{17}H_{28}O_2$ 264.41	Practically insoluble or insoluble 1 ml in 1 ml	158 (10.7 hPa) 156 MS 95 %	n.a. n.a.	
09.356	1,1-Dimethylethyl propionate		20487-40-5	Liquid $C_7H_{14}O_2$ 130.19	Practically insoluble or insoluble 1 ml in 1 ml	118 MS 95 %	1.390-1.396 0.862-0.868	
09.614	Linalyl valerate		10738 10471-96-2	Liquid $C_{15}H_{26}O_2$ 238.37	Practically insoluble or insoluble 1 ml in 1 ml	238 MS 95 %	1.450-1.456 0.897-0.903	Racemate.
09.617	p-Menthan-8-yl acetate		58985-18-5	Liquid $C_{12}H_{22}O_2$ 198.29	Practically insoluble or insoluble 1 ml in 1 ml	115 (13 hPa) MS 95 %	1.449-1.455 0.930-0.940	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation18, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.669	Myrcenyl acetate		10857 1118-39-4	Liquid $C_{12}H_{20}O_2$ 196.29	Practically insoluble or insoluble 1 ml in 1 ml	112 (13 hPa) MS 95 %	1.456-1.462 0.915-0.921	
09.671	Nerolidyl acetate		10862 56001-43-5	Liquid $C_{17}H_{28}O_2$ 264.41	Practically insoluble or insoluble 1 ml in 1 ml	107 (0.4 hPa) MS 95 %	1.467-1.473 0.901-0.907	(3S,6Z)-isomer. Name to be changed accordingly.
09.808	Guaiyl acetate		10659 134-28-1	Solid $C_{17}H_{28}O_2$ 264.41	Practically insoluble or insoluble 1 ml in 1 ml	269 96 MS 95 %	n.a. n.a.	

1) *Solubility in water, if not otherwise stated.*
2) *Solubility in 95 % ethanol, if not otherwise stated.*
3) *At 1013.25 hPa, if not otherwise stated.*
4) *At 20°C, if not otherwise stated.*
5) *At 25°C, if not otherwise stated.*
6) *Stereoisomeric composition not specified.*
7) *AV: Missing minimum assay value.*
8) *BP: Missing boiling point.*

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)

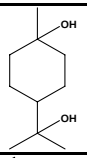
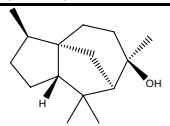
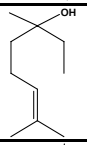
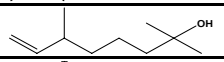
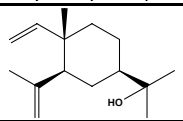
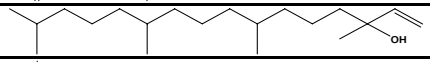
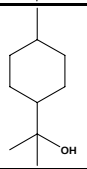
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.054	p-Menthane-1,8-diol		11	Class I A3: Intake below threshold	4)	6)	
02.120	Cedrol		13	Class I A3: Intake below threshold	4)	6)	
02.140	1,2-Dihydrolinalool		0.044	Class I A3: Intake below threshold	4)	6)	
02.144	2,6-Dimethyloct-7-en-2-ol		0.0012	Class I A3: Intake below threshold	4)	6)	
02.149	Elemol		1.6	Class I A3: Intake below threshold	4)	6)	
02.168	Isophytol		0.037	Class I A3: Intake below threshold	4)	7)	
02.171	p-Menthan-8-ol		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)

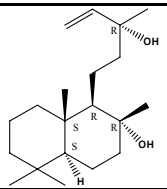
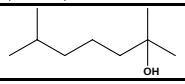
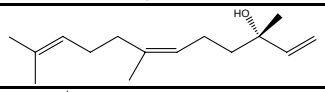
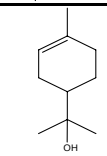
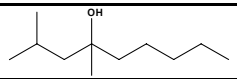
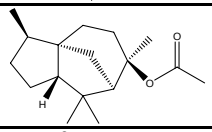
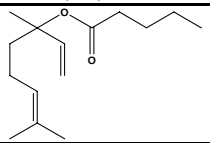
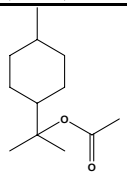
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.206	Sclareol		0.67	Class I A3: Intake below threshold	4)	6)	
02.219	2,6-Dimethyl-2-heptanol		0.012	Class I A3: Intake below threshold	4)	6)	
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol		0.049	Class I A3: Intake below threshold	4)	6)	
02.230	Terpineol	 alfa-Terpineol shown	1200	Class I A3: Intake below threshold	4)	6)	
02.253 1850	2,4-Dimethyl-4-Nonanol		0.24	Class I A3: Intake below threshold	4)	6)	
09.171	Cedryl acetate		0.99	Class I A3: Intake below threshold	4)	6)	
09.614	Linalyl valerate		0.43	Class I A3: Intake below threshold	4)	6)	
09.617	p-Menthan-8-yl acetate		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)

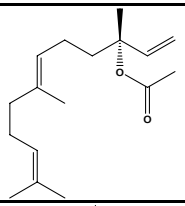
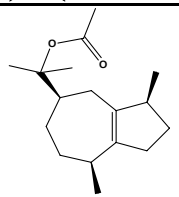
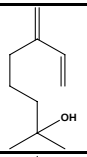
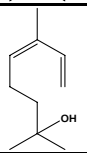
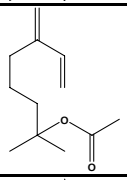
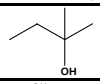
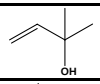
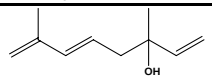
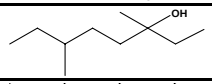
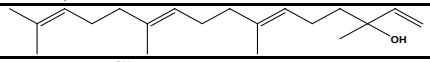
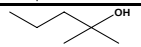
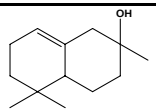
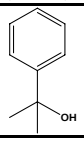
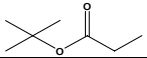
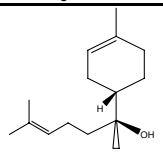
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.671	Nerolidyl acetate		0.061	Class I A3: Intake below threshold	4)	6)	
09.808	Guaiyl acetate		0.0012	Class I A3: Intake below threshold	4)	6)	
02.185	Myrcenol		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
02.191	Ocimenol		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
09.669	Myrcenyl acetate		8.6	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
02.041	2-Methylbutan-2-ol		2.7	Class II A3: Intake below threshold	4)	6)	
02.052	2-Methylpropan-2-ol		0.012	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
02.123	2-Methylbut-3-en-2-ol		0.0012	Class II A3: Intake below threshold	4)	6)	
02.146	3,7-Dimethylocta-1,5,7-trien-3-ol		12	Class II A3: Intake below threshold	Additional data required		
02.147	3,6-Dimethyloctan-3-ol		0.0012	Class II A3: Intake below threshold	4)	7)	
02.150	Geranyl linalool		0.026	Class II A3: Intake below threshold	4)	6)	
02.181	2-Methylpentan-2-ol		0.12	Class II A3: Intake below threshold	4)	6)	
02.184	3-Methylpentan-3-ol		0.0012	Class II A3: Intake below threshold	4)	6)	
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol		0.026	Class II A3: Intake below threshold	4)	7)	
02.203	2-Phenylpropan-2-ol		0.0012	Class II A3: Intake below threshold	4)	6)	
09.356	1,1-Dimethylethyl propionate		0.0012	Class II A3: Intake below threshold	4)	6)	
02.129	Bisabola-1,12-dien-8-ol	 (l)-alpha-Bisabolol shown	27	Class III A3: Intake below threshold	4)	7)	

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

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

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

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

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

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

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

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

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

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

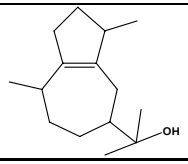
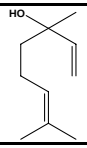
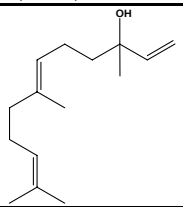
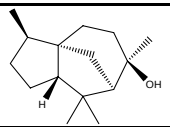
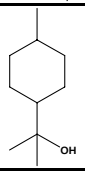
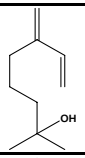
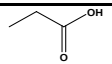
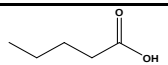
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Guaiol		Not evaluated as flavour		Not in EU-Register
02.013	Linalool 356		No safety concern a) Category A b)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
02.018	Nerolidol 1646		Category B b)	Class I A3: Intake below threshold	
02.052	2-Methylpropan-2-ol		Category B b) FGE.18	Class II A3: Intake below threshold	FGE.18: 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.
02.120	Cedrol		FGE.18	Class I A3: Intake below threshold	
02.171	p-Menthan-8-ol		FGE.18	Class I A3: Intake below threshold	FGE.18: No safety concern based on the intake calculation by the MSDI approach.

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.185	Myrcenol		FGE.18	Class I B3: Intake below threshold, B4: No adequate NOAEL	FGE.18: Additional data requested.
08.002	Acetic acid 81		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.003	Propionic acid 84		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 c) No safety concern d) Category A b)	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 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.*

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

a) (JECFA, 2000a).

b) (CoE, 1992).

c) (SCF, 1995).

d) (JECFA, 1999b.)

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

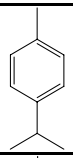
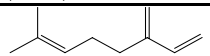
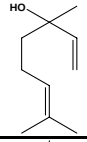
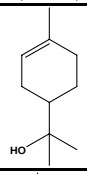
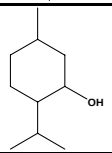
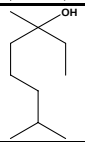
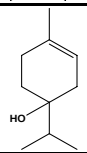
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
01.002	1-Isopropyl-4-methylbenzene		2356 620 99-87-6	1325 JECFA specification (JECFA, 2005b)	926	No safety concern a) Category B b)	
01.008	Myrcene		2762 2197 123-35-3	1327 JECFA specification (JECFA, 2005b)	290	No safety concern a) Category B b)	EFSA conclusion: B4-No, additional data required (EFSA, 2009j).
02.013	Linalool		2635 61 78-70-6	356 JECFA specification (JECFA, 1998b)	2200	No safety concern c) Category A b)	GrADI: 0-0.5 (JECFA, 1980a)
02.014	alpha-Terpineol		3045 62 98-55-5	366 JECFA specification (JECFA, 1998b)	2600	No safety concern c) Category A b)	
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998b)	16000	No safety concern c) Category A b)	ADI: 0-4 (JECFA, 2000a).
02.028	3,7-Dimethyloctan-3-ol		3060 77 78-69-3	357 JECFA specification (JECFA, 1998b)	47	No safety concern c) Category B b)	
02.072	4-Terpinenol		2248 2229 562-74-3	439 JECFA specification (JECFA, 2000d)	150	No safety concern c) Category B b)	

Table 3: Supporting Substances Summary

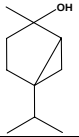
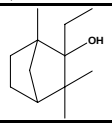
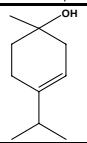
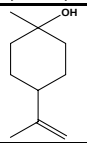
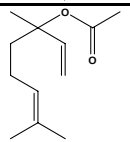
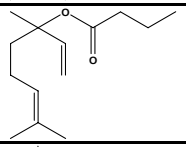
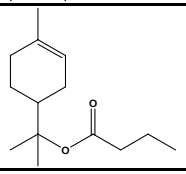
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.085	Sabinene hydrate		3239 10309 546-79-2	441 JECFA specification (JECFA, 2000d)	0.91	No safety concern c)	
02.095	2-Ethylfenchol		3491 10208 18368-91-7	440 JECFA specification (JECFA, 2001c)	0.74	No safety concern c)	
02.096	1-Terpinenol		3563 10252 586-82-3	373 JECFA specification (JECFA, 2000d)	35	No safety concern c)	
02.097	beta-Terpineol		3564 10254 138-87-4	374 JECFA specification (JECFA, 2001c)	1.3	No safety concern c)	
09.013	Linalyl acetate		2636 203 115-95-7	359 JECFA specification (JECFA, 1998b)	1700	No safety concern c) Category A b)	GrADI: 0-0.5 (JECFA, 1980a).
09.050	Linalyl butyrate		2639 276 78-36-4	361 JECFA specification (JECFA, 1998b)	8.4	No safety concern c) Category A b)	
09.052	Terpinyl butyrate		3049 278 2153-28-8	370 JECFA specification (JECFA, 2001c)	5.1	No safety concern c) Category A b)	

Table 3: Supporting Substances Summary

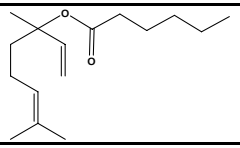
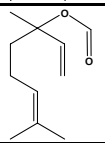
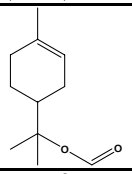
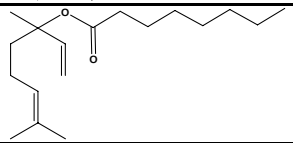
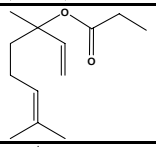
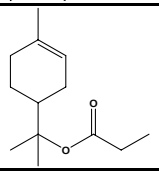
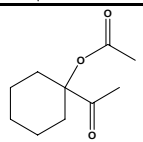
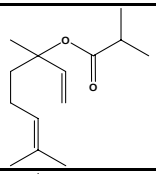
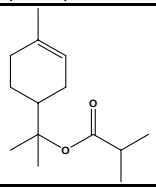
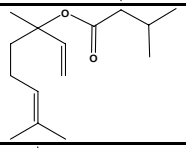
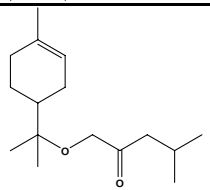
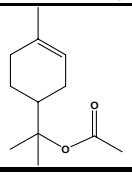
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.068	Linalyl hexanoate		2643 318 7779-23-9	364 JECFA specification (JECFA, 1998b)	0.85	No safety concern c) Category A b)	
09.080	Linalyl formate		2642 347 115-99-1	358 JECFA specification (JECFA, 2003b)	6.9	No safety concern c) Category A b)	GrADI: 0-0.5 (JECFA, 1980a).
09.081	alpha-Terpinyl formate		3052 348 2153-26-6	367 JECFA specification (JECFA, 2001c)	0.12	No safety concern c) Category A b)	
09.116	Linalyl octanoate		2644 397 10024-64-3	365 JECFA specification (JECFA, 2000d)	0.12	No safety concern c) Category B b)	
09.130	Linalyl propionate		2645 411 144-39-8	360 JECFA specification (JECFA, 2003b)	13	No safety concern c) Category A b)	
09.142	Terpinyl propionate		3053 423 80-27-3	369 JECFA specification (JECFA, 1998b)	0.024	No safety concern c) Category B b)	
09.293	1-Acetoxy-1-acetylcyclohexane		3701 52789-73-8	442 JECFA specification (JECFA, 2001c)	ND	Additional data required c)	Additional data required (JECFA, 2000a).

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
09.423	Linalyl isobutyrate		2640 298 78-35-3	362 JECFA specification (JECFA, 1998b)	30	No safety concern c) Category A b)	
09.425	Terpinyl 2-methylpropionate		3050 300 7774-65-4	371 JECFA specification (JECFA, 2000d)	0.61	No safety concern c) Category B b)	
09.454	Linalyl isovalerate		2646 449 1118-27-0	363 JECFA specification (JECFA, 2000d)	4.6	No safety concern c) Category B b)	
09.461	Terpinyl isovalerate		3054 456 1142-85-4	372 JECFA specification (JECFA, 2001c)	0.12	No safety concern c) Category B b)	
09.830	Terpineol acetate		3047 205 8007-35-0	368 JECFA specification (JECFA, 1998b)	220	No safety concern c)	

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

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

3) No safety concern at estimated levels of intake.

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

a) (JECFA, 2005c).

b) (CoE, 1992).

c) (JECFA, 2000a).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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

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

- can the flavourings be predicted to be metabolised to innocuous products[8] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[9] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

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

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

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

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

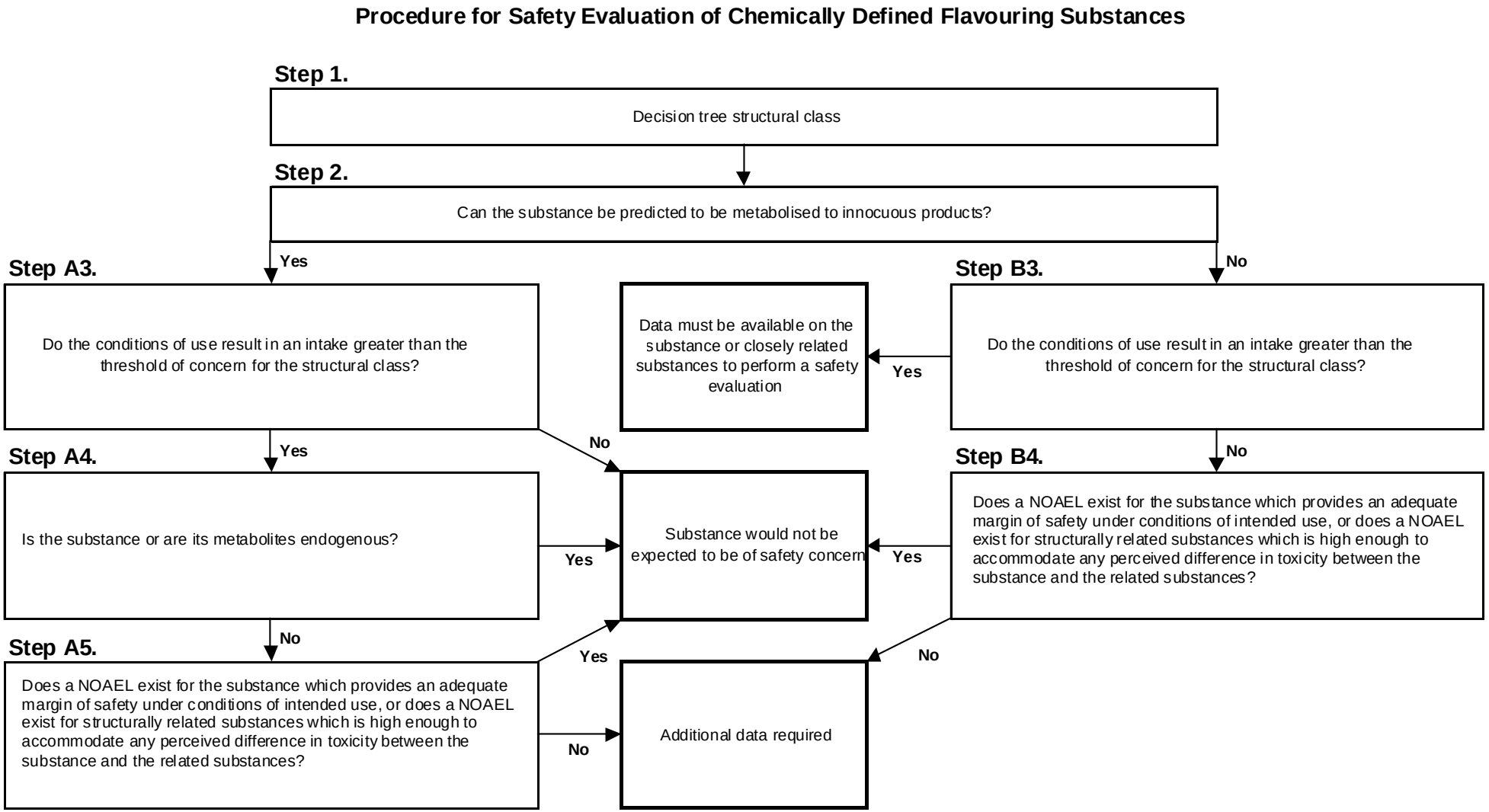


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

The "normal and maximum use levels" are provided by Industry for 31 of the 32 candidate substances in the present flavouring group (EFFA, 2004a; EFFA, 2005e; EFFA, 2006k; EFFA, 2007a; EFFA, 2007h; Flavour Industry, 2009c) (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18, Revision 2 (EFFA, 2004a; EFFA, 2005e; EFFA, 2006k; EFFA, 2007a; EFFA, 2007h; Flavour Industry, 2009c).

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
02.041	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.052	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.054	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.120	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.123	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.129	10	10	8	10	-	20	10	4	-	-	-	-	20	-	10	10	-	10
	50	50	50	30	-	100	30	20	-	-	-	-	100	-	30	30	-	500
02.140	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.144	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.147	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18, Revision 2 (EFFA, 2004a; EFFA, 2005e; EFFA, 2006k; EFFA, 2007a; EFFA, 2007h; Flavour Industry, 2009c).

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
02.149	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.150	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.168	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.171	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.181	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.184	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.185	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.191	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-
02.203	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.206	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.219	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.226	2	-	2	-	-	200	2	2	-	-	-	-	1	-	2	30	2	-
	8	-	80	-	-	500	7	6	-	-	-	-	5	-	5	100	10	-
02.230	20	10	50	-	-	50	-	50	5	-	-	-	50	-	10	60	50	-
	150	50	300	-	-	300	-	300	10	-	-	-	300	-	300	300	300	-
02.253	7	5	10	7	-	10	5	10	2	-	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	-	-	-	25	50	25	50	100	25
09.171	7	5	10	7	7	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	35	50	25	50	10	10	-	-	25	50	25	50	100	2
09.356	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.614	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.617	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.669	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.671	7	5	-	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	-	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.808	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

II.2 mTAMDI Calculations

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

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

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0

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)
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 (EC) No1565/2000	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	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 the 31 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004a; EFFA, 2005e; EFFA, 2006k; EFFA, 2007a; EFFA, 2007h; Flavour Industry, 2009c). The mTAMDI values are only given for highest reported normal use levels (See Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.054	p-Menthane-1,8-diol	3900	Class I	1800
02.120	Cedrol	3900	Class I	1800
02.140	1,2-Dihydrolinalool	3900	Class I	1800
02.144	2,6-Dimethyloct-7-en-2-ol	3900	Class I	1800
02.149	Elemol	3900	Class I	1800
02.168	Isophytol	3900	Class I	1800
02.171	p-Menthan-8-ol	3900	Class I	1800
02.206	Sclareol	3900	Class I	1800
02.219	2,6-Dimethyl-2-heptanol	3900	Class I	1800
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	7000	Class I	1800
02.230	Terpineol	14000	Class I	1800
02.253	2,4-Dimethyl-4-Nonanol	3900	Class I	1800
09.171	Cedryl acetate	3900	Class I	1800
09.614	Linalyl valerate	3900	Class I	1800
09.617	p-Menthan-8-yl acetate	3900	Class I	1800
09.671	Nerolidyl acetate	3900	Class I	1800
09.808	Guaiyl acetate	3900	Class I	1800
02.185	Myrcenol	3900	Class I	1800
02.191	Ocimenol	3900	Class I	1800
09.669	Myrcenyl acetate	3900	Class I	1800
02.041	2-Methylbutan-2-ol	3900	Class II	540
02.052	2-Methylpropan-2-ol	3900	Class II	540
02.123	2-Methylbut-3-en-2-ol	3900	Class II	540
02.146	3,7-Dimethylocta-1,5,7-trien-3-ol		Class II	540
02.147	3,6-Dimethyloctan-3-ol	3900	Class II	540
02.150	Geranyl linalool	3900	Class II	540
02.181	2-Methylpentan-2-ol	3900	Class II	540
02.184	3-Methylpentan-3-ol	3900	Class II	540
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	1600	Class II	540
02.203	2-Phenylpropan-2-ol	3900	Class II	540
09.356	1,1-Dimethylethyl propionate	3900	Class II	540
02.129	Bisabola-1,12-dien-8-ol	5700	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

A consideration of the chemical structures of the candidate substances in this Flavouring Group Evaluation, their anticipated pathways of metabolism and the extent to which data on one substance may support the metabolism of another substance has indicated that it is appropriate to divide the candidate substances in this evaluation into eight subgroups of more closely related structures (See Table III.1.).

Table III.1. Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
1	02.041	2-Methylbutan-2-ol	Aliphatic saturated tertiary alcohols and one ester thereof
	02.052	2-Methylpropan-2-ol	
	02.147	3,6-Dimethyloctan-3-ol	
	02.181	2-Methylpentan-2-ol	
	02.184	3-Methylpentan-3-ol	
	02.219	2,6-Dimethyl-2-heptanol	
	02.253	2,4-Dimethyl-4-nonanol	
	09.356	1,1-Dimethylethyl propionate	
2	02.123	2-Methylbut-3-en-2-ol	Aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two esters thereof
	02.144	2,6-Dimethyloct-7-en-2-ol	
	02.150	Geranyl linalool	
	02.168	Isophytol	
	02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	
	09.614	Linalyl valerate	
	09.671	Nerolidyl acetate	
3	02.146	3,7- Dimethylocta-1,5,7-trien-3-ol	Aliphatic unsaturated tertiary alcohols with conjugated terminal double bonds and one ester thereof
	02.185	Myrcenol	
	02.191	Ocimenol	
	09.669	Myrcenyl acetate	
4	02.140	1,2-Dihydrolinalool	Aliphatic unsaturated tertiary alcohol (without terminal double bond)
5	02.054	p-Menthane-1,8-diol	Monocyclic saturated and unsaturated tertiary alcohols and one ester thereof
	02.129	Bisabola-1,12-dien-8-ol	
	02.171	p-Menthan-8-ol	
	02.230	Terpineol	
	09.617	p-Menthan-8-yl acetate	
6	02.149	Elemol	Monocyclic and bicyclic unsaturated tertiary alcohols with isolated terminal double bonds
	02.206	Sclareol	
7	09.808	Guaiyl acetate	Bi- and tricyclic tertiary alcohols and esters
	02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnapthalen-2-ol	
	02.120	Cedrol	
	09.171	Cedryl acetate	
8	02.203	2-Phenylpropan-2-ol	Tertiary alcohol with an aromatic substituent

III.2. Absorption, Distribution and Elimination

Specific information on absorption, distribution and excretion is available for the candidate substances, 2-methylpropan-2-ol (tertiary butanol) [FL-no: 02.052], elemol [FL-no: 02.149], sclareol [FL-no: 02.206] and terpineol [FL-no: 02.230].

2-Methylpropan-2-ol [FL-no: 02.052] was administered by gastric intubation to female Wistar rats at a single dose of 1853 mg/kg bw and blood concentrations were monitored for 20 hours. 2-Methylpropan-2-ol was eliminated slowly from the blood of rats in this study, with concentrations in blood at 2, 5 and 20 hours measured as 13.2, 12.6 and 11.4 mM, respectively (Beaugé et al., 1981). The high bolus dose of 2-methylpropan-2-ol may have exceeded the capacity of the metabolic pathways for detoxication and contributed to the relatively slow clearance time from the blood.

The pharmacokinetic profile for 2-methylpropan-2-ol was characterised in male (four/dose) and female (three/dose) F-344 rats following intravenous administration of 37.5, 75, 150 and 300 mg 2-methylpropan-2-ol/kg bw. Blood samples were collected in heparinised syringes at 5, 10, 20, 30, 40 and 60 min, and 4, 8, 12, 16 and 24 hours from the cannula implanted in the right jugular vein of each animal. The data fit a two-compartment model with the distribution half-life (T½ α) about 3 min and the elimination half-life (T½ β) about 3.8 hours in male and female rats for doses less than 300 mg/kg bw. Consistent with another study (Beaugé et al., 1981), the elimination of 2-methylpropan-2-ol appears to get saturated at higher doses, as evidenced by a disproportional increase in area under the concentration – time curve and decreased rate of clearance in this study. After administration of a 300 mg/kg bw dose of 2-methylpropan-2-ol, the means of T½ β were increased to 5.0 and 4.3 hours in male and female rats, respectively. The steady-state volume of distribution for 2-methylpropan-2-ol was approximately 4.5-fold greater than total body water, suggesting significant tissue distribution (Poet et al., 1997).

Potential pharmacokinetic outcomes in mammalian plasma were analysed by intravenous administration of sclareol [FL-no: 02.206] at 100 mg/kg bw to two male Wistar rats. Plasma samples were collected at 5, 15, 30, 60, 180, 360, 720 and 1440 min after administration, and sclareol concentrations were quantified by gas-liquid chromatography using an internal standard. At 5 min post-injection, plasma levels of sclareol were 84.9 µg/ml. The sclareol plasma concentration dropped to 42.9 µg/ml after 180 min, and was not detectable at 720 min. This study indicated a rapid biphasic disappearance of sclareol from plasma following intravenous treatment. The authors suggest that sclareol may be distributed in fatty tissue due to its high lipophilicity. Sclareol was administered to two male Wistar rats by intravenous administration at 100 mg/kg bw, and to male Wistar rats by oral gavage at 1 g/kg bw in 3:1 propylene glycol-ethanol. Urine and faecal samples were collected from all rats at periodic intervals over 144 or 72 hours, respectively. Bile samples were collected only from rats given intravenous injections at periodic intervals over 30 hours. No sclareol was detected in urine or urine treated with β-glucuronidase at any time. No sclareol was detected in faecal samples from intravenous treated rats, but 9 % of the initial sclareol dose was found in faecal samples from rats given oral administration. The bile samples from rats given intravenous administration showed very low levels (0.02 %) of sclareol over a 3 hours period. Very low levels (0.04 %) of oxidised metabolites were found in bile after 3 hours, including 3-alpha-hydroxysclareol (0.24 %), 3-betahydroxysclareol (0.075 %), 18-hydroxysclareol (0.056 %), and 3-ketosclareol (0.03 %). Sclareol or its oxidised metabolites were not observed in bile samples collected at any other time during the 30 hours study. The authors hypothesized that the low sensitivity of the assay technique may have prevented detection of low levels of sclareol and its metabolites over the course of the experiment. While only a very small percentage of intravenously administered sclareol (<0.05 %) could be accounted for in these experiments, the authors suggested that other mammalian metabolites were formed that were not detected in the assays used (Kouzi et al., 1993).

Incubation of sclareol with 37 different microorganisms found that sclareol is oxidised to more polar metabolites, including 3-ketosclareol, 2α-hydroxysclareol, 3β-hydroxysclareol, 18-hydroxysclareol, 2α,18-dihydroxysclareol, and three glucoside conjugates (Kouzi et al., 1993).

Intravenous administration of sclareol to rats, however, found very low levels of the hydroxylated and glycosidic metabolites or the parent compound in the urine.

A metabolism study on the structurally related elemol [FL-no: 02.149] indicates that substances of this type are absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of this candidate substance would not be anticipated.

alpha-Terpineol, the major component of terpineol [FL-no: 02.230], undergoes allylic oxidation of the exocyclic methyl group, which can then be further oxidised to a carboxylic acid group (Madyastha & Srivatsan, 1988b). In a minor pathway, the doublebond of alpha-terpineol is epoxidized and then hydrolysed to yield the triol metabolite 1,2,8-trihydroxy-p-menthane, which also has been reported in humans following inadvertent oral ingestion of a pine oil disinfectant containing alpha-terpineol (Horning et al., 1976). It is expected that after single dose exposures, alpha terpineol would undergo metabolism via glucuronic acid conjugation and excretion in the urine (Chadha & Madyastha, 1984; Hill et al., 1975; Wright, 1945).

(-)-Elemol (2 g) was orally given to rabbits (2-3 kg) and the urine was collected for the three following days (72 hours). In total 80 % of the administered dosage was recovered from the urine (Asakawa et al., 1986).

In addition, there is information on absorption and excretion for the four supporting substances linalool, myrcene, menthol and 1-isopropyl-4-methylbenzene (synonym: p-cymene).

[1,2-^{14}C]-Linalool was orally administered to rats at a single dose of 500 mg/kg bw. The majority (55 %) of the radioactivity was excreted in the urine as the glucuronic acid conjugate, whereas 23 % was excreted as CO_2 in expired air, and 15 % was excreted in the faeces within 72 hours of administration. Only 3 % of the radioactivity was detected in tissues after 72 hours, with 0.5 % in the liver, 0.6 % in the gut, 0.8 % in the skin and 1.2 % in the skeletal muscle (Parke et al., 1974a).

When given to male Japanese White rabbits by gavage at a dose of 670 mg/kg bw per day for two days, approximately 25 % of the total administered amount (19 g to six rabbits) of myrcene could be recovered from the urine excreted over a period of three days following administration (Ishida et al., 1981).

In humans 79 % of a 1000 mg oral dose (Quick, 1928b) or 78 % of a 10 to 20 mg oral dose (Atzl et al., 1972) of menthol administered to volunteers was eliminated as the glucuronic acid conjugate. For eight days 750 mg l-menthol was administered orally to two human volunteers followed by oral or intravenous administration of 200 mg [6-^{13}C]-glucuronolactone or [6-^{13}C]-sodium glucuronate. For two days after administration of the isotopic compound, menthyl glucuronide was excreted in an average daily yield ranging from approximately 27 % to 84 % of the l-menthol administered (Eisenberg et al., 1955). Four males were given an oral dose of 180 mg peppermint oil. Between 37 and 116 mg menthol glucuronide were excreted in the urine after 14 hours (Kaffenberger & Doyle, 1990).

Non-cannulated and bile duct-cannulated male Fischer 344 rats were administered a single oral dose of 500 mg [3-3H]-1-menthol/kg bw. Urine and faeces were collected over the next 24 and 48 hours from non-cannulated rats. In the bile duct-cannulated rats, bile samples were collected at 2-hours intervals for the first 6 hours and then from 6 to 24 hours. The 0-24 hours urine was collected in the same rats. In the bile duct-cannulated rats, total recovery of the dose of the radiolabeled substance in the urine or bile was 74.2 % after 24 hours. The amount of radioactivity found in the urine after 24 hours from the non-cannulated and the bile duct-cannulated rats differed from 19 to 7.3 %, but indicates that most of the compound is excreted in the bile during the first 24 hours (Yamaguchi et al., 1994).

Of an oral dose of 100 mg/kg bw of p-cymene given to male Wistar rats or Dunkin Hartley guinea pigs, 80 % or 71 %, respectively, was excreted in the urine within the following 48 hours in the form of extractable

metabolites. It was speculated that the rest of the dose was either excreted via the faeces or as unextractable metabolites in the urine (Walde et al., 1983).

In conclusion, the candidate substance 2-methylpropan-2-ol is anticipated to be absorbed and to undergo rapid excretion when administered at lower doses. However, when administered at higher doses the metabolic capacity may be exceeded. The candidate substances elemol and sclareol are absorbed but excreted rather slowly. The candidate substance terpineol is anticipated to be absorbed and excreted. The supporting substances, menthol and p-cymene, are readily absorbed and excreted rapidly with approximately 70 % recovery within 24 hours and 70-80 % recovery within 48 h, respectively. Linalool is absorbed but rather slowly excreted. The slowest absorption and excretion was observed for myrcene where only 25 % of administered dose was recovered in urine after 72 h.

III.3. Biotransformation

III.1 Ester hydrolysis

Aliphatic esters are hydrolysed to the component alcohols and carboxylic acid by carboxyl-esterases found in most tissues throughout the body, the most important of which are the beta-esterases (Heymann, 1980). In mammals these enzymes occur within the body in most tissues including the gut lumen and intestinal wall, but predominate in the hepatocytes (Heymann, 1980). The wide range of tissue distribution and the multiplicity of the esterases generally give rapid hydrolyse of esters *in vivo*.

No hydrolysis studies on the candidate esters [FL-no: 09.171, 09.356, 09.614, 09.669, 09.671, 09.808 and 09.617] are available. Two hydrolysis studies on a supporting substance, linalyl acetate [FL-no: 09.013], were found. In an *in vitro* hydrolysis study, linalyl acetate was easily hydrolysed in water and simulated gastric and pancreatic fluids. The mean half-lives for linalyl acetate hydrolysis were 5.5 and 52.5 min in gastric and pancreatic fluids, respectively (Hall, 1979). In neutral gastric juice, linalyl acetate is slowly ($t_{½}$ = 121 min) hydrolysed to a mixture of linalool and the ring-closed isomer alpha-terpineol. In acidic artificial gastric juice, linalyl acetate is rapidly hydrolysed ($t_{½}$ < 5 min) to yield linalool (Buck & Renwick, 1998). Linalyl acetate was slowly hydrolysed ($t_{½}$ = 153 – 198 min) in intestinal fluid with or without pancreatin. Linalyl acetate also hydrolyses in homogenates of rat intestinal mucosa, blood and liver, but at rates much slower than in acidic gastric juice (rate constant for hydrolysis, k = 0.01 to 0.0055 min^{-1} vs. > 5 min^{-1} in gastric juice). Based on these observations it can be concluded that linalyl acetate hydrolyses in gastric juice to yield linalool and acetic acid (Buck & Renwick, 1998). Further, it has been demonstrated that linalyl acetate can be hydrolysed to linalool in *in vitro* studies after incubation with rat caecal flora (Rahman, 1974a).

III.2 Subgroup 1 - Aliphatic saturated tertiary alcohols and ester

For three of the eight candidate substances in this subgroup, 2-methylpropan-2-ol [FL-no: 02.052], 2-methylbutan-2-ol [FL-no: 02.041] and 2-methylpentan-2-ol [FL-no: 02.181], there are one or more metabolism studies.

2-Methylpropan-2-ol (tert-Butanol [FL-no: 02.052])

[2-^{13}C]-2-Methylpropan-2-ol was orally administered in a gel capsule at a dose of 5 mg/kg bw to one human volunteer (44 year old, 80 kg male). Urine was collected in 12-hours intervals for 48 hours and analysed by ^{13}C-NMR. All of the urine samples of this human volunteer showed the presence of 2-methylpropan-2-ol, 2-methylpropan-2-ol glucuronide, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol. In contrast to the rat urine samples in which 2-methylpropan-2-ol sulphate was observed as a major metabolite, the sulphate was only present in trace amounts in the human volunteer urine samples. The low recovery of 2-methylpropan-2-ol sulphate in the urine of the human volunteer is likely based on a low affinity of human sulphotransferase

for 2-methylpropan-2-ol as compared to rats. On the other hand, 2-hydroxyisobutyrate was the major metabolite excreted by the human volunteer. The likely pathway for the formation of the 2-hydroxyisobutyrate and 2-methyl-1,2-propanediol metabolites involves methyl group oxidation of 2-methylpropan-2-ol by CYP-450 to yield 2-methyl-1,2-propanediol. Further oxidation of 2-methyl-1,2-propanediol results in the formation of 2-hydroxyisobutyrate, and acetone is likely formed by further oxidation of the intermediate diol and/or 2-hydroxyisobutyrate (Bernauer et al., 1998).

Three male rats per experiment were administered a single 250 mg/kg bw dose of [12C]- or [13C]- 2-methylpropan-2-ol dissolved in corn oil by oral gavage. All animals were maintained in individual metabolism cages for 72 hours, after which they were sacrificed by cervical dislocation. Urine was collected at 24 and 48 hours and analysed by 13C-NMR. In the 24-hours urine samples, it was determined that 2-methylpropan-2-ol sulphate, 2-methyl-1,2-propanediol, and 2-hydroxyisobutanoate (i.e. alpha-hydroxyisobutanoate) are the major metabolites of 2-methylpropan-2-ol in rats; minor metabolites were identified as free 2-methylpropan-2-ol, 2-methylpropan-2-ol glucuronide, and [13C]-acetone. Identical metabolites were present at lower concentrations in the urine collected between 24 and 48 hours after dosing. Extensive biotransformation of tert-butyl alcohol in rats by conjugation and oxidation was confirmed by the results of these experiments (Bernauer et al., 1998).

2-Methylpropan-2-ol was administered by gastric intubation to chinchilla rabbits at a 297 mg/kg bw. 2-Methylpropan-2-ol was conjugated to a large extent with glucuronic acid, and conjugates were readily isolated from urine. Of the administered dose, 24.4 % was excreted as glucuronic acid conjugate in the urine within 24 hours after administration. The investigators suggested that the volatile alcohol may also be eliminated to some extent via the lungs. No aldehydes or ketones were detected in the expired air of a rabbit administered 6 ml of 2-methylpropan-2-ol (Kamil et al., 1953a).

2-Methylbutan-2-ol (tert-amyl-alcohol [FL-no: 02.041])

2-Methylbutan-2-ol was administered by gastric intubation to chinchilla rabbits at a single dose of 441 mg/kg bw. 2-Methylbutan-2-ol was conjugated to a large extent with glucuronic acid and 58 % of the dose was excreted as such conjugates via the urine within 24 hours post dosing (Kamil et al., 1953a).

Three male rats were administered a single 250 mg/kg bw dose of [2-13C]-2-methylbutan-2-ol dissolved in corn oil by oral gavage. All animals were maintained in individual metabolism cages for 48 hours. Urine was collected at 24 and 48 hours and analysed by ^{13}C-NMR. In the 24-hour urine samples, it was determined that 2-methylbutan-2-ol glucuronide, 2-methyl-2,3-butanediol and 2-methyl-2,3-butanediol glucuronide are the major metabolites of 2-methylbutan-2-ol; minor metabolites were identified as free 2-methylbutan-2-ol, 2-hydroxy-2-methylbutanoic acid and 3-hydroxy-3-methylbutanoic acid. The low concentration of 2-methylbutan-2-ol recovered in the urine suggests extensive metabolism of the alcohol. In the 48-hour urine samples, only 2-methyl-2,3-butanediol and 2-methyl-2,3-butanediol glucuronide were detected, suggesting the rapid excretion of 2-methylbutan-2-ol glucuronide following oral exposure. Glucuronidation appears to be the major pathway of metabolism, resulting in urinary excretion of the 2-methylbutan-2-ol glucuronide. In addition, it appears that 2-methylbutan-2-ol is oxidised to 2-methyl-2,3-butanediol, which is further conjugated to 2-methyl-2,3-butanediol glucuronide and excreted in the urine. A minor metabolic pathway seems to be oxidation of the carbon atom at the C4 position resulting in 2-methyl-2,4-butanediol as an intermediate, which is further oxidised to 3-hydroxy-3-methylbutanoic acid. Finally, oxidation of the methyl side chain is another minor transformation pathway that results in the formation of 2-methyl-1,2-butanediol as an intermediate, which is further oxidised to produce 2-hydroxy-2-methylbutanoic acid (Amberg et al., 1999) (See Figure III.1.)

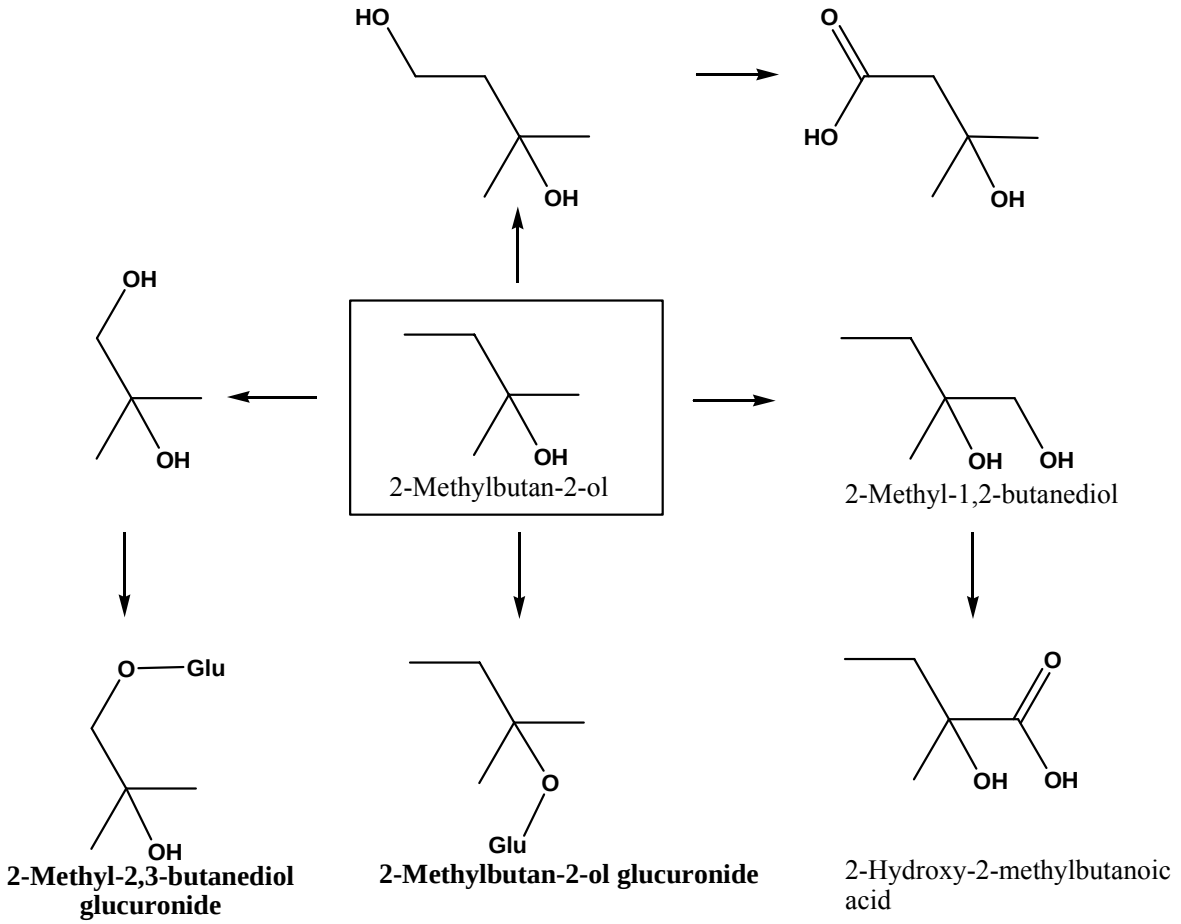


Figure III.1. *Metabolism of 2-methybutan-2-ol (tert-amyl-alcohol) in rats (Amberg et al., 1999). Main metabolism products are in bold.*

2-Methylpentan-2-ol (tert-hexylalcohol [FL-no: 02.181])

2-Methylpentan-2-ol was administered by gastric intubation to chinchilla rabbits at a single dose of 851 mg/kg bw. 2-Methylpentan-2-ol was conjugated to a large extent with glucuronic acid, and 57 % of the dose was excreted within 24 hours post dosing as such conjugates via the urine (Kamil et al., 1953a).

III.3 Subgroup 2 - Aliphatic unsaturated tertiary alcohols and esters with isolated terminal double bonds

There are no metabolism studies on the candidate substances 2-methylbut-3-en-2-ol, 2,6-dimethyloct-7-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate, [FL-no: 02.123, 02.144, 02.150, 02.168, 02.226, 09.614 and 09.671]. However, there is a metabolism study for linalool, supporting substance to 2-methylbut-3-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate [FL-no: 02.123, 02.150, 02.168, 02.226, 09.614 and 09.671,], which have the isolated double bond in close proximity to the tertiary alcohol group.

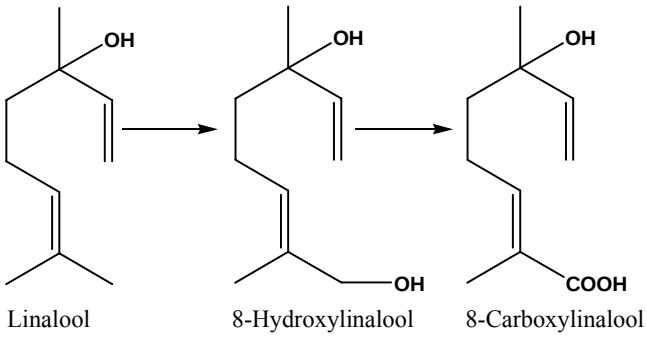


Figure III.2 *Metabolism of linalool (Chadha & Madyastha 1984).*

Seventy-two hours after intragastrical administration of 500 mg/kg bw ^{14}C-labelled linalool/kg bw to 12-weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetra hydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut (Rahman, 1974a).

In another study, male rats were given a daily 800 mg/kg bw oral dose of linalool for 20 days. Urinary metabolites formed by CYP-450-mediated allylic oxidation of linalool included 8-hydroxylinalool and 8-carboxylinalool (Chadha & Madyastha, 1984) (Figure III.2). No oxidation of the terminal double bond was observed, indicating no formation of epoxide intermediates.

No metabolism studies on supporting substances to 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144], which has the isolated double bond far from the tertiary alcohol group, are available.

III.4 Subgroup 3: Aliphatic unsaturated tertiary alcohols and ester with conjugated terminal double bonds

No metabolism studies are found for the candidate substances, 3,7-dimethylocta-1,5,7-trien-3-ol, myrcenol, ocimenol or myrcenyl acetate [FL-no: 02.146, 02.185, 02.191 and 09.669]. For the supporting substance, myrcene, two studies on metabolism are found.

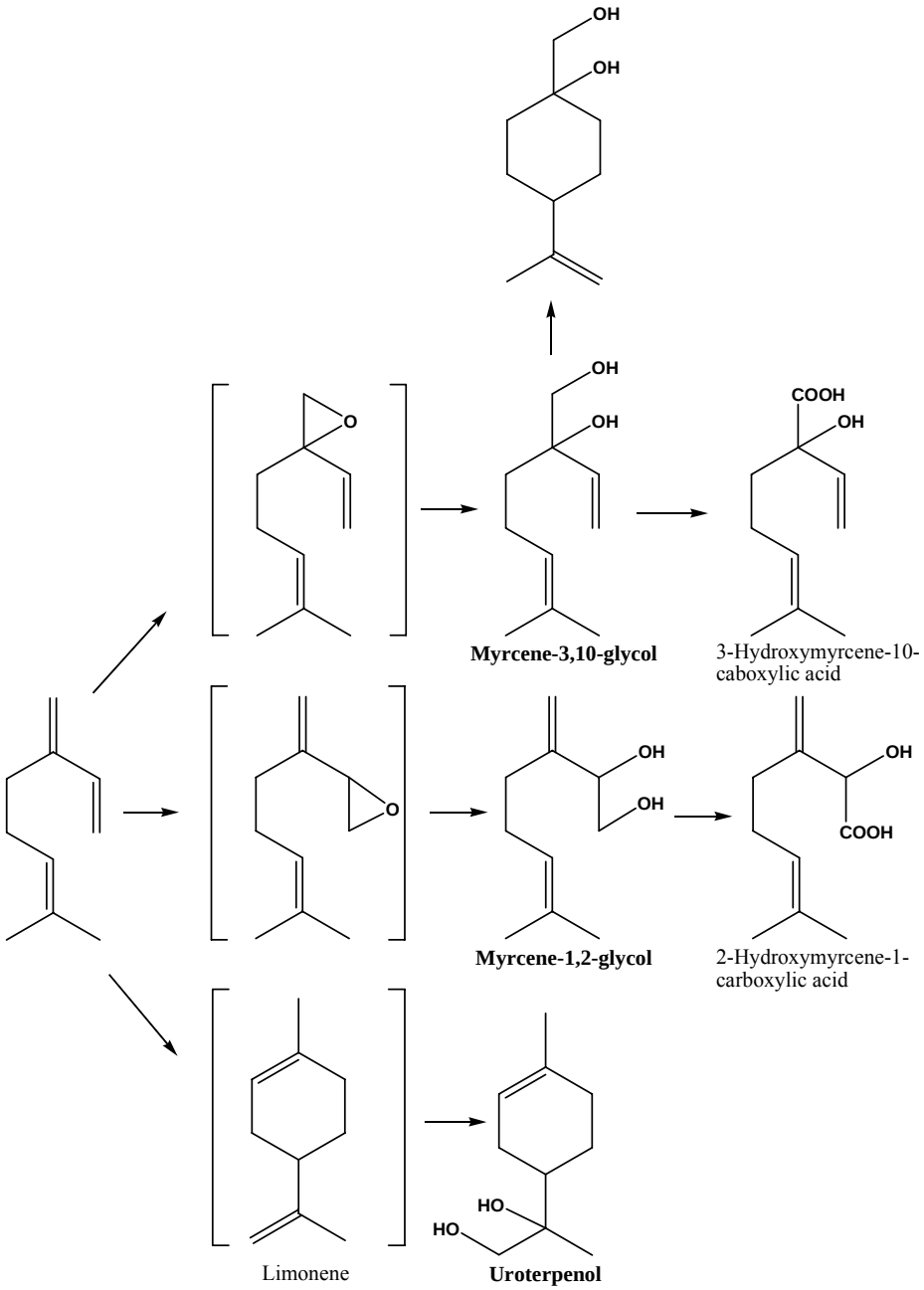


Figure III.3 *Metabolism of myrcene (Ishida et al., 1981 and Madyastha & Srivatsan 1987). Intermediate products are in brackets and main metabolism products are in bold.*

In the urine of rabbits orally administered myrcene (single dosage of 670 mg/kg bw) via gavage, more than 80 % of the metabolites were neutral metabolites, the rest were acidic metabolites. The main metabolites identified in urine were myrcene-3,10-glycol, myrcene-1,2-glycol and uroterpenol (40.7, 20.8 and 11.8 %, respectively, of the neutral metabolites after 72 hours). Additionally, the glycols underwent further oxidation to yield 2-hydroxymyrcene-1-carboxylic acid and 3-hydroxymyrcene-10-carboxylic acid (no quantitative data were given for these acidic metabolites).

The authors suggested that uroterpenol (or limonene-8,9-diol) may have been formed from limonene, which is derived from cyclization of myrcene in the acidic conditions of the rabbit stomach (Ishida et al., 1981).

When rats were administered 800 mg/kg bw myrcene per day via gavage for 20 days, the principal metabolites isolated from the urine were 10-hydroxylinalool (or myrcene-3,10-glycol) and, to a lesser extent, 7-methyl-3-methylene-oct-6-ene-1,2-diol (or myrcene-1,2-glycol). Other minor metabolites included the hydroxy acids of both the 3,10- and 1,2-glycols (10-carboxylinalool (or 3-hydroxymyrcene-10-carboxylic acid) and 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid (or 2-hydroxymyrcene-1-carboxylic acid), respectively) and a cyclic diol, 1-hydroxymethyl-4-isopropenylcyclohexanol (or p-menth-8-ene-1,7-diol), formed by intramolecular cyclization of an open chain metabolite (Madyastha & Srivatsan, 1987).

It was demonstrated that the biotransformation of myrcene was cytochrome P450 (CYP)-mediated and that it could be enhanced by pretreatment of animals with phenobarbital (Madyastha & Srivatsan, 1987). These studies indicate the oxidation of the terminal double bonds of myrcene via intermediate epoxidation of the 1,2- and 3,10- double bonds (Figure III.3).

III.5 Subgroup 4: Aliphatic unsaturated tertiary alcohols

After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep, dihydrolinalool and tetrahydrolinalool are formed as metabolites (Rahman, 1974a). *In vivo* metabolism studies in rats on ^{14}C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucoronides (Rahman, 1974a).

III.6 Subgroup 5: Monocyclic saturated tertiary alcohols and esters

There are no metabolism studies on the candidate alicyclic substances p-menthane-1,8-diol, bisabola-1,12-dien-8-ol, p-menthan-8-ol, terpineol and p-menthan-8-yl acetate [FL-no: 02.054, 02.129, 02.171, 02.230 and 09.617]. p-Menthan-8-yl acetate is anticipated to be hydrolysed to give p-menthan-8-ol. There are metabolism studies on two substances, alpha-terpinol and menthol, which are supporting substances to p-menthane-1,8-diol, p-menthan-8-ol, and p-menthan-8-yl acetate (Figure III.4).

In a repeated dose study, male albino rats were orally administered the alicyclic tertiary alcohol alpha-terpineol at a daily dose of 600 mg/kg bw for 20 days. Oxidation of the allylic methyl group was observed to yield the corresponding carboxylic acid, which was hydrogenated, to a small extent, to yield the corresponding saturated carboxylic acid (Madyastha & Srivatsan, 1988b).

In rats, the vast majority of orally administered menthol is eliminated in either the urine or faeces as the glucuronic acid or various oxidation products (Madyastha & Srivatsan, 1988b; Yamaguchi et al., 1994).

Non-cannulated and bile duct-cannulated male Fischer 344 rats were given a single oral dose of 500 mg/kg bw [3-3H]-1-menthol. Urine and faeces were collected over the next 24 and 48 hours from non-cannulated rats. In the bile duct-cannulated rats, bile samples were collected at 2-hours intervals for the first 6 hours and then from 6 to 24 hours. The 0-24 hours urine was collected in the same rats (Yamaguchi et al., 1994). The major metabolites found were menthol glucuronide in the bile and a variety of oxidation products in the urine. Menthol glucuronide formed in the liver passes into the bile with subsequent elimination or entry into enterohepatic circulation. Oxidation products of menthol are primarily p-menthane-3,8-diol, 3,8-dihydroxy-p-menthane-7-carboxcylic acid and 3-hydroxy-p-menthane-7-carboxylic acid (Figure III.4). Minor metabolites are p-menthane-3,7-diol, p-menthane-3,7,8-triol, p-menthane-3,9-diol and 3-hydroxy-p-menthane-9-carboxylic acid. The monohydroxylated menthols are excreted in the urine in part as glucuronide (Yamaguchi et al., 1994).

The study by Madyastha & Srivatsan (Madyastha & Srivatsan, 1988b) supports the finding of p-menthane-3,8-diol and 3,8-dihydroxy-p-menthane-7-carboxcylic acid as the main metabolites of menthol in urine after giving rats an oral dosage of 800 mg/kg bw. They also found p-menthane-3,9-diol as a minor metabolite.

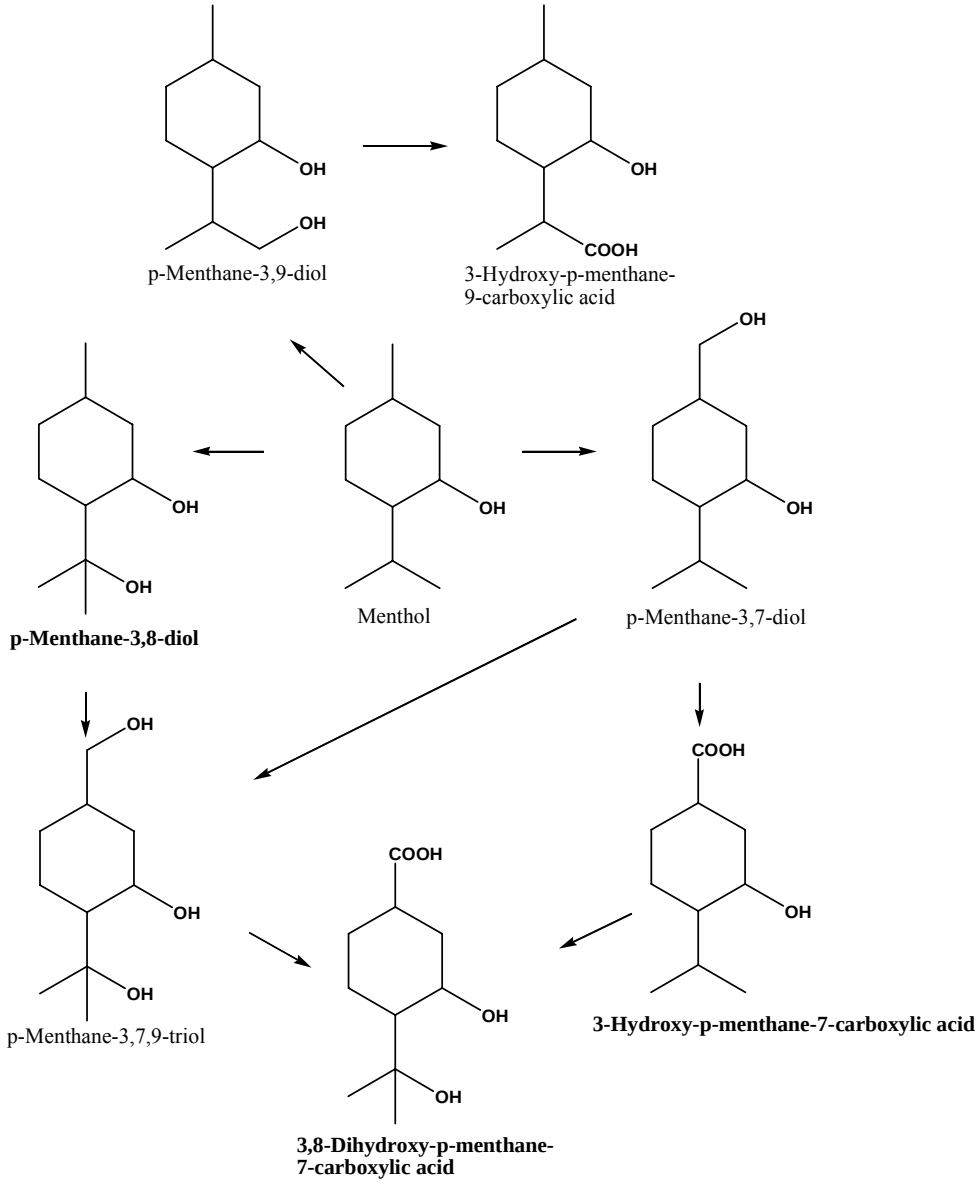


Figure III.4. *Metabolism of menthol in rats (Yamaguchi et al., 1994). Main metabolism products are in bold.*

III.7 Subgroup 6: Monocyclic unsaturated tertiary alcohols with isolated terminal double bonds

There is one metabolism study on one of the two candidate substances in this group, elemol [FL-no: 02.149]. (-)-15-Hydroxyelemol was the only metabolite (10 % of the natural metabolites) detected after 72 hours in the urine of rabbits (2-3 kg) given 2g (-)-elemol. Most of the (-)-elemol (70 %) was recovered as elemol conjugated with glucuronic acid or sulphate. No oxidation of the double bond of the vinyl group was observed, indicating that no epoxide intermediates was formed.

III.8 Subgroup 7: Bi- and tricyclic tertiary alcohols and esters

For the candidate substances guaiyl acetate [FL-no: 09.808], 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphtalen-2-ol [FL-no: 02.197] and cedryl acetate [FL-no: 09.171] there are no metabolism studies available. Two metabolism studies were found for the candidate substance cedrol [FL-no: 02.120]. In the study by Bang and Ourisson (1975) rabbits were given natural cedrol (1 g) suspended in a 1 % mathyl-

cellulose mucilage (20 ml) administered by gavage followed by 25 ml water. The urine was collected at 24, 48 and 96 hours after administration. The urine was acidified to pH 4.5 and hydrolysed at 37° C for 24 hours with 6 ml snail (D-glucuronide)-glucuronidase. Five percent of the dose was excreted as conjugation product of unchanged cedrol. Thirtyfive percent of the dose was recovered as a mixture of stereochemically different 3-hydroxycedrol metabolites. Twelve percent of the dose was recovered as a C7-C8 dehydrated C3-hydroxylation product of cedrol (both 3-R and 3-S) (Bang & Ourisson, 1975).

Trifilieff et al. (1975) administered 2 grams of cedrol to a phenobarbital-pretreated fasted dog. The metabolites described below (see also Figure III.5) were found in the first 24 hours urine. Urine was acidified with hydrochloric acid and treated with glucuronidase-sulphatase to hydrolyse conjugates. Three metabolite fraction, isolated by chromatography on silica columns and acetylation, were obtained in the following amounts: 130 mg of diol (1), 100 mg of a mixed fraction of diols (2 plus 3), and 40 mg of an acid (4). Diol (1) was identified by spectral data and correlation with α-epi-isobiotol (= 3-hydroxycedrol) and the corresponding ketone. In contrast to the rabbit the dog produced only the 3S isomer. Diols (2 plus 3) were identified as 15-hydroxycedrol and 2-hydroxycedrol, respectively, by separation after dehydration and further derivation and identification of the reaction products by comparison of the spectral data with literature data. The acid (4) was identified as the C14 carboxylic acid derivation product of 2-hydroxycedrol. In total only 270 mg (13 % of the dose) was recovered, but elimination of cerdol (-conjugates) was not studied / reported (Trifilieff et al., 1975). For structures of cedrol and the four metabolites in the dog see scheme below (Figure III.5):

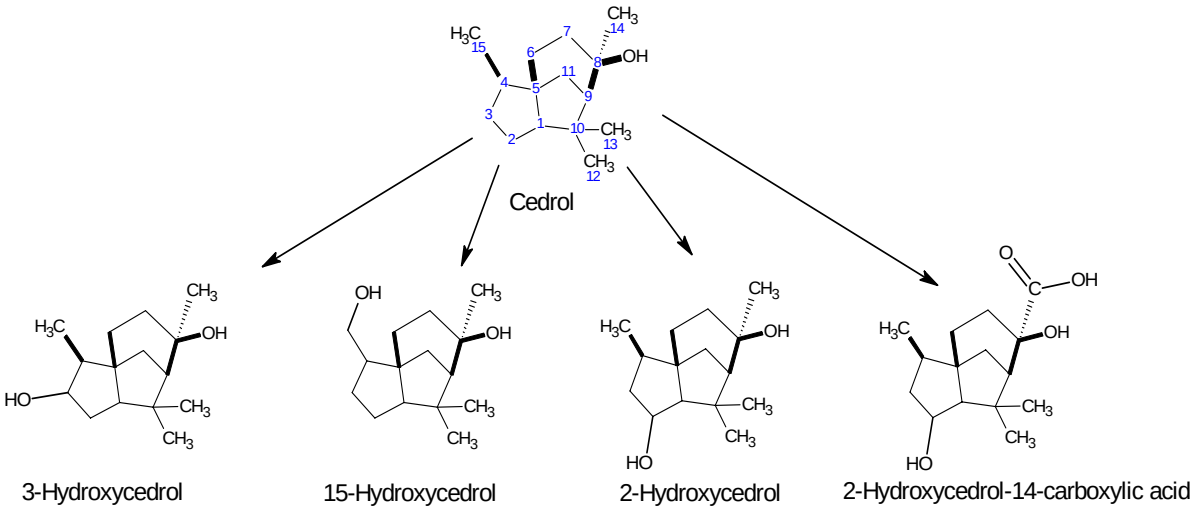


Figure III.5 *Metabolism of cedrol in a dog treated with phenobarbital. The carboxylic acid is presumably formed from 2-hydroxycedrol, but this was not further studied (after Trifilieff et al, 1975).*

III.9 Subgroup 8: A aromatic tertiary alcohols

No metabolism studies were found for the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203]. However, several metabolism studies were found for the supporting substance p-cymene.

The main metabolites in the urine of rabbits given a single oral dose of 670 mg p-cymene /kg bw were p-cymen-9-ol and p-cymen-8-ol (50 % and 28 %, respectively, of the neutral metabolites). Acidic metabolites identified were alpha-p-tolylpropionic acid, alpha-tolyl-alpha-hydroxylpropionic acid, p-isopropylbenzoic acid and p-1-hydroxyisopropylbenzoic acid. Ring hydroxylation did not occur (Ishida et al., 1981).

Following an oral dose of 100 mg/kg bw of p-cymene to male rats, the principal urinary metabolites were p-isopropylbenzoic acid (19 % of the administered dose) and 2-p-carboxyphenylpropionic acid (16 %). Other less important urinary metabolites included 2-p-tolylpropan-1-ol (8 %), 2-p-tolylpropan-2-ol (9 %), 2-p-carboxyphenylpropan-2-ol (9 %), 2-p-(hydroxymethyl)phenylpropionic acid (4 %), 2-p-carboxyphenylpropan-1-ol (11 %), p-isopropylbenzoylglycine (2 %), p-isopropylbenzyl alcohol (1 %), and 2-p-tolylpropionic acid (1 %) (Walde et al., 1983). When the same dose was given to male guinea pigs, similar urinary metabolites were identified, however in different quantities. The primary urinary metabolite in guinea pigs was p-isopropylbenzoylglycine (31 %), indicating that conjugation with glycine was more prevalent in guinea pigs than in rats. Another major metabolite in guinea pigs was 2-p-tolylpropan-2-ol (14 %). In addition, whereas ring hydroxylation of p-cymene was not reported in rats (Bakke & Scheline, 1970; Walde et al., 1983) and rabbits (Ishida et al., 1981), trace amounts of the ring hydroxylation metabolites hydroxyl-p-cymene and hydroxycarvacrol (dehydroxyl-p-cymene) were detected in guinea pig urine. Ring hydroxylation in guinea pigs only occurred in ortho position to the methyl group (Walde et al., 1983).

Boyle et al. (1999) studied the metabolite pattern of p-cymene in rats following oral doses equivalent to 50 and 200 mg/kg bw. The major metabolites in 0-48 hours urine after administration of the 50 mg/kg bw dose were 2-p-tolypropan-2-ol (39 % of recovered dose) and 2-p-carboxphenylpropan-2-ol (19 %). The former metabolite is the product of allylic hydroxylation of the isopropyl substituent, while the latter metabolite is the product of allylic hydroxylation of both the isopropyl substituent and the methyl substituent. Minor metabolites in rat urine were 2-p-carboxyphenylpropan-1-ol (10 %), 2-p-carboxyphenylpropionic acid (14 %), and p-isopropylbenzoic acid (17 %). A large percentage of the urinary metabolites at this dose was conjugated (66 % conjugated vs 34 % free) both to glucuronic acid and glycine. The same metabolites were observed after the high dose, but conjugation was considerably reduced (18 % conjugated vs 82 % free), suggesting saturation of the conjugation pathway (Boyle et al., 1999).

III.10 Oxidation of terminal double bonds

13 candidate substances [FL-no: 02.123, 02.168, 02.144, 02.146, 02.185, 02.191, 02.150, 02.206, 02.226, 09.614, 09.669, 09.671 and 02.149] possess terminal double bonds. These double bonds may be oxidised to the corresponding epoxide. Epoxides are highly reactive molecules, due to the large strain associated with the three membered ring structures, and they react easily with nucleophilic sites of cellular macromolecules. For this reason, several aliphatic alkene-derived epoxides have been demonstrated to be carcinogenic (Melnick, 2002).

Two of the main metabolites of one of the supporting substances, myrcene, were found to be produced through epoxidation of the double bonds followed by hydrolysis to diols by epoxide hydroxylase (see above). Theoretically these reactions may also occur in the candidate substances with conjugated terminal double bonds. However, linalool or elemol were not found to give oxidation of terminal double bounds, indicating that terminal double bonds as such will not give rise to intermediate epoxides.

III.4. Summary and Conclusions

Seven candidate flavouring substances in this group are esters [FL-no: 09.171, 09.356, 09.614, 09.669, 09.671, 09.808 and 09.617]. Hydrolysis data are not available for any of these esters. However, for the supporting substance, linalyl acetate, *in vitro* hydrolysis data indicate that these esters may be hydrolysed, which would also be expected based on general knowledge about ester hydrolysis. The carboxcylic acids resulting from the hydrolysis of these six candidate flavouring substances are acetic acid, propanoic acid and valeric acid, which will be incorporated in normal physiological processes such as beta-oxidation and citric acid cycle. The alcohols resulting from the hydrolysis of these esters are tertiary alcohols as are all the remaining candidate substances in this flavouring group. The tertiary alcohols in this FGE are subdivided into eight subgroups according to their chemical structures.

Subgroup 1: Metabolism studies of the three candidate substances, 2-methylpropan-2-ol [FL-no: 02.052], 2-methylbutan-2-ol [FL-no: 02.041] and 2-methylpentan-2-ol [FL-no: 02.181] show that these are conjugated with glucuronic acid before excretion in the urine. When rats were treated with 2-methylpropan-2-ol, acetone was excreted in small amounts, and when administered 2-methylbutan-2-ol, diols were excreted. This indicates that an additional metabolism pathway of the three candidate substances is oxidation of the methyl group. From these metabolism studies it is anticipated that the candidate substances 2-methylpropan-2-ol, 2-methylbutan-2-ol, 3-methylpenta-3-ol, 2-methylpentan-2-ol, 2,6-dimethyl-2-heptanol, 2,4-dimethyl-4-nonanol, 3,6-dimethyloctan-3-ol and 1,1-dimethylethyl propionate [FL-no: 02.052, 02.041, 02.184, 02.181, 02.219, 02.253, 02.147 and 09.356] are conjugated with glucuronic acid and excreted in the urine, or that they can undergo oxidation to yield the corresponding diols. They are also expected to be excreted as their respective glucuronic acid conjugates.

Subgroup 2: Linalool is a supporting substance to the candidate substances 2-methylbut-3-en-2-ol, geranyl linalool, isophytol, [S-(cis)]-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, linalyl valerate and nerolidyl acetate [FL-no: 02.123, 02.150, 02.168, 02.226, 09.614 and 09.671,], which have the isolated double bond in close proximity to the tertiary alcohol group. As these substances or their respective alcohol moieties have a free hydroxyl group, they may be directly conjugated. Seventy-two hours after intragastrical administration of 500 mg/kg bw 14C-labelled linalool to 12-weeks old rats 58-60 % of the dose was excreted in the urine, 12-15 % in the faeces and 25-27 % in the expired air. In tissues 3-4 % residual activity was found. Beyond unchanged linalool the main metabolites in urine and faeces were dihydrolinalool and tetra hydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut. In addition, the metabolism of linalool indicates that these candidate substances may also be metabolised by omega-oxidation of methyl groups, excreted in the urine as such or in conjugation with glucuronic acid. No oxidation of the terminal double bond in linalool was observed, indicating no formation of epoxide intermediates.
For 2,6-dimethyloct-7-en-2-ol [FL-no: 02.144] the structure differs from the supporting substance linalool and the other candidate substances in this group in that the isolated terminal double bond is located distant from the tertiary group. However, any risk from epoxide formation of this compound is considered to be low since the substance can be directly conjugated with glucuronic acid or the alcohol group can be expected to be readily attacked by oxidation processes, ultimately yielding the corresponding carboxylic acid. Biochemical attack of this carboxylic acid via beta-oxidation or conjugation with glucuronic acid is expected to be more efficient and rapid than microsomal oxidation. Any epoxides formed may be metabolised by conjugation with glutathione or by epoxide-hydrolase mediated hydrolysis.

Subgroup 3: Myrcene is a supporting substance to the candidate substances 3,7-dimethylocta-1,5,7-trien-3-ol, myrcenol, ocimenol and myrcenyl acetate [FL-no: 02.146, 02.185, 02.191 and 09.669]. These substances also have alcohol moieties that can be directly conjugated. In addition, further oxidation of methyl groups may occur. As shown for myrcene, oxidation of conjugated terminal double bonds in the candidate substances may occur, giving rise to epoxide intermediates. It should be noted that the available genotoxicity data for myrcene do not indicate a genotoxic potential for this substance even in the presence of metabolic activation. However, it cannot be anticipated that these candidate substances will be metabolised to innocuous products.

Subgroup 4: 1,2-Dihydrolinalool [FL-no: 02.140] can be expected to be directly conjugated to glucuronic acid like the supporting substance linalool, and excreted. After incubation of linalool or linalyl acetate with gut microflora from rat, mice or sheep, dihydrolinalool and tetrahydrolinalool are formed as metabolites. *In vivo* metabolism studies in rats on 14C-labelled linalool demonstrated that linalool can be metabolised to dihydrolinalool and further to tetrahydrolinalool in the gut and excreted in urine and faeces as sulphates and glucoronides. Additionally, it might be oxidised at the methyl groups, introducing new hydroxyl groups that also can be conjugated and excreted.

Subgroup 5: From the metabolism studies of alpha-terpineol and menthol it is anticipated that the candidate substances terpineol [FL-no: 02.230], p-menthane-1,8-diol [FL-no: 02.054], bisabola-1,12-dien-8-ol [FL-no:

02.129] and p-menthan-8-ol [FL-no: 02.171] (also an hydrolysis product of candidate substance [FL-no: 09.617]) may undergo allylic oxidation of the exocyclic methyl group. This could be further oxidised to a carboxylic acid group. Alternative or subsequent metabolism may occur by conjugation to glucuronic acid, followed by excretion in the urine.

Subgroup 6: A metabolism study on elemol indicate that the substance is absorbed from the gastrointestinal tract and mainly excreted in conjugation with glucuronic acid or sulphate, although one oxidised metabolite, hydroxyelemol, was also found in lower amounts. No oxidation of the isolated terminal double bond of elemol was found; accordingly, epoxidation of elemol [FL-no: 02.149] and sclareol [FL-no: 02.206], which have the same structural features as elemol, would not be anticipated.

Subgroup 7: Two metabolism studies on cedrol [FL-no: 02.120] indicate that the candidate substances 1,2,3,4,4a,5,6,7-octahydro-2,5,5-trimethylnaphthalen-2-ol [FL-no: 02.197], cedrol [FL-no: 02.120] and cedryl acetate [FL-no: 09.171] and guaiyl acetate [FL-no: 09.808] will be further hydroxylated and excreted in urine as such or as conjugates.

Subgroup 8: In metabolism studies, the supporting substance 1-isopropyl-4-methylbenzene [FL-no: 01.002] (synonym: p-cymene) was oxidised at the isopropyl side chain yielding 2-(p-tolyl)-2-propanol, which is not further oxidised, but excreted unchanged or as a glucuronic acid conjugate. It is anticipated that the candidate substance 2-phenylpropan-2-ol [FL-no: 02.203] will follow the same pathway and be excreted unchanged or in conjugation with glucuronic acid.

In summary, 28 of the candidate substances [FL-no: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.171, 02.181, 02.184, 02.197, 02.203, 02.206, 02.219, 02.226, 02.230, 02.253, 09.808, 09.171, 09.356, 09.614, 09.617 and 09.671] are anticipated to be metabolised to innocuous products.

Four of the candidate substances [FL-no: 02.146, 02.185, 02.191 and 09.669] (subgroup 3) contain conjugated terminal double bonds and data from a supporting substance indicate that these may be oxidised, giving rise to epoxide intermediates. Thus, it cannot be anticipated that these four substances will be metabolised to innocuous products. Despite evidence for the formation of epoxide intermediates, the supporting substance produced negative results in *in vitro* genotoxicity studies.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 16 candidate substances of the present Flavouring Group Evaluation from chemical group 6 and 8, and for 19 supporting substances evaluated by the JECFA at the 51st meeting (JECFA, 1999a). The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Rat	NR	Oral	3500	(Schaffarzick & Brown, 1952)	
	Rat	M, F	Gavage	3046	(Johnson, 1981a)	
	Rat	M, F	Gavage	2733	(Johnson, 1981b)	
	Rat	NR	Oral	> 3800	(Eastman Kodak Co., 1994c)	
	Rabbit	M, F	Gavage	3560	(Munch, 1972)	
2-Methylbutan-2-ol [02.041]	Rat	NR	Oral	1000	(Schaffarzick & Brown, 1952)	
	Rat	NR	Oral	1000 – 2000	(Dow Chemical Company, 1982b)	
	Rabbit	M, F	Gavage	2027	(Munch, 1972)	
3-Methylpentan-3-ol [02.184]	Rat	NR	Oral	710	(Brown et al., 1955)	
2,6-Dimethyl-2-heptanol [02.219]	Rat	NR	Oral	6800	(BASF, 1979b)	
	Rat	NR	Oral	> 5000	(Moreno, 1976a)	
3,6-Dimethyloctan-3-ol [02.147]	Rat	NR	Oral	> 5000	(Moreno, 1973n)	
2-Methylbut-3-en-2-ol [02.123]	Rat	NR	Oral	1800	(BASF, 1972)	
(Linalool [02.013])	Rat	M, F	Gavage	2790	(Jenner et al., 1964)	
	Mouse	M, F	Oral	2200	(Rhône-Poulenc, Inc., 1992a)	
	Mouse	M, F	Oral	3918	(Hoffman-LaRoche, Inc., 1967b)	
	Mouse	M, F	Oral	3000[1] 1700[2]	(Rhône-Poulenc, Inc., 1992a)	
(3,7-Dimethyloctan-3-ol [02.028])	Rat	NR	Oral	> 5000	(Moreno, 1976r)	
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat	NR	Oral	3600	(Moreno, 1973o)	
Myrcenol [02.185]	Rat	NR	Oral	5300	(Moreno, 1972g)	
Ocimenol [02.191]	Rat	NR	Oral	1700	(Wohl, 1974f)	
Geranyl linalool [02.150]	Rat	NR	Oral	> 5000	(Moreno, 1982i)	
	Mouse	M, F	Oral	14,632	(Hoffman-LaRoche, Inc., 1967a)	
(Linalyl formate [09.080])	Rat	NR	Oral	> 5000	(Russell, 1973e)	
	Mouse	M	Oral	5389	(Hoffman-LaRoche, Inc., 1967b)	
(Linalyl acetate [09.013])	Rat	M, F	Gavage	14,550	(Jenner et al., 1964)	
	Rat	NR	Oral	10,000	(Zeller, 1969)	
	Mouse	NR	Gavage	13,360	(Jenner et al., 1964)	
	Mouse	M	Oral	13,539	(Hoffman-LaRoche, Inc., 1967b)	
(Linalyl propionate [09.130])	Rat	NR	Oral	> 5000	(Moreno, 1973p)	
	Mouse	M	Oral	13,874	(Hoffman-LaRoche, Inc., 1967b)	
(Linalyl butyrate [09.050])	Rat	NR	Oral	> 5000	(Levenstein, 1975i)	
	Mouse	M	Oral	> 8907	(Hoffman-LaRoche, Inc., 1967b)	
(Linalyl isobutyrate [09.423])	Rat	M, F	Gavage	> 36,300	(Jenner et al., 1964)	
	Mouse	NR	Gavage	15,100	(Jenner et al., 1964)	
	Mouse	M	Oral	> 17,698	(Hoffman-LaRoche, Inc., 1967b)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Linalyl isovalerate [09.454])	Rat	NR	Oral	> 5000	(Moreno, 1975l)	
	Mouse	M, F	Oral	25,165	(Hoffman-LaRoche, Inc., 1967b)	
(Linalyl hexanoate [09.068])	Mouse	M, F	Oral	37,869	(Hoffman-LaRoche, Inc., 1967a)	
Myrcenyl acetate [09.669]	Rat	NR	Oral	6300	(Moreno, 1972h)	
(Linalyl octanoate [09.116])	Mouse	M, F	Oral	48,849	(Hoffman-LaRoche, Inc., 1967a)	
Nerolidyl acetate [09.671]	Rat	NR	Oral	> 5000	(Moreno, 1976s)	
(alpha-Terpineol [02.014])	Rat	NR	Oral	4300[3]	(Moreno, 1971)	
	Mouse	M	Gavage	2830	(Yamahara et al., 1985)	
(alpha-Terpinyl formate [09.081])	Rat	NR	Oral	> 5000	(Moreno, 1976t)	
(Terpinyl acetate [09.830])	Rat	M, F	Gavage	5075	(Jenner et al., 1964)	
(Terpinyl propionate [09.142])	Rat	NR	Oral	> 5000	(Moreno, 1973q)	
(Terpinyl 2-methylpropionate [09.425])	Rat	NR	Oral	> 5000	(Moreno, 1982j)	
(4-Terpinenol [02.072])	Rat	NR	Oral	1300	(Moreno, 1977v)	
Guaiyl acetate [09.808]	Rat	NR	Oral	> 5000	(Moreno, 1973r)	
2-Phenylpropan-2-ol [02.203]	Rat	NR	Oral	1037[4]	(Patty, 1982a)	
(beta-Terpineol) [02.097])	Rat	NR	Oral	4300[3]	(Moreno, 1971)	
p-Menthan-8-ol [02.171]	Rat	NR	Oral	> 5000	(Moreno, 1973s)	
(1-Acetoxy-1-acetylcyclohexane [09.293])	Rat	M, F	Gavage	2150	(Piccirillo & Hartman, 1980b)	
(Menthol [02.015])	Mouse	M	Gavage	2652	(Food and Drug Research Laboratories, Inc., 1975a)	
	Mouse	M	Gavage	4384	(Food and Drug Research Laboratories, Inc., 1975a)	
	Mouse	NR	Gavage	3100	(Wokes, 1932)	
	Rat	M, F	Gavage	3180	(Jenner et al., 1964)	
	Rat	M	Gavage	940	(Food and Drug Research Laboratories, Inc., 1975a)	
Bisabola-1,12-dien-8-ol [02.129]	Rat	NR	Oral	> 5000	(CIR, 1999)	
	Mice	NR	Oral	0.633	(CIR, 1999)	
	Rat	M, F	Oral	M: 14,9 ml/kg bw F: 15.6 ml/kg bw	(Habersang et al., 1979)	
	Mice	M, D	Oral	15.1 ml/kg bw	(Habersang et al., 1979)	

[1]*Value represents calculated LD_{50} when substance was administered in peanut oil.*

[2]*Value represents calculated LD_{50} when substance was administered as an emulsion in an aqueous Arabic gum solution at 10 %.*

[3]*Reported for a mixture of alpha- and beta-terpineol.*

[4]*LD_{50} value reported as 1.07 ml; conversion based on a density of 0.97 g/ml.*

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for six candidate substances of the present Flavouring Group Evaluation from chemical group 6 and 8, and for eight supporting substances evaluated by the JECFA at the 51st meeting (JECFA, 1999a) or at the 63rd meeting (JECFA, 2006a). The supporting substances are listed in brackets.

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

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
2-Methylpropan-2-ol [02.052]	Rat; M 5-6	Drinking water	0 or 0.5 %, equivalent to 0 or 500 mg/kg bw/day	10 weeks	None	(Acharya et al., 1997)	Histological examination of liver and kidney only.
	Rat; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw/day	90 days	None	(Lindamood et al., 1992; NTP, 1995b)	Fully described NTP study. No NOAEL for males or females.
	Rat; M 10	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw/day	90 days	None	(Takahashi et al., 1993)	Good quality investigative study using extra renal samples from the above 90-day study; indicates nephropathy in male F344 rats is due to alpha-2 µ-globulin. No NOAELs.
	Rat; M, F 120[4]	Drinking water	Males: 0, 0.125, 0.25 or 0.5 %, equivalent to 0, 90, 200 or 420 mg/kg bw/day Females: 0, 0.25, 0.5 or 1 %, equivalent to 0, 180, 330 or 650 mg/kg bw/day	2 years	None	(Cirvello et al., 1995; NTP, 1995b)	Fully described NTP study. No NOAELs. NTP conclusions on carcinogenicity: some evidence of carcinogenic activity in males, no evidence of carcinogenicity in females.
	Rat; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 250, 500, 1000, 2000 or 4000 mg/kg bw/day	90 days	Male: 0.5 %, equivalent to 500 mg/kg bw/day Female: 1 %, equivalent to 1000 mg/kg bw/day	(Brown & Wheeler, 1979)	The report is incomplete as it is lacking tables.
	Mouse; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 625, 1250, 2500, 5000 or 10,000 mg/kg bw/day	90 days		(Brown & Wheeler, 1979)	The report is incomplete as it is lacking tables.
	Mouse; M, F 20	Drinking water	0, 0.25, 0.5, 1, 2 or 4 %, equivalent to 0, 625, 1250, 2500, 5000 or 10,000 mg/kg bw/day	90 days	Male: 1 %, equivalent to 2500 mg/kg bw/day Female: 2 %, equivalent to 5000 mg/kg bw/day	(Lindamood et al., 1992; NTP, 1995b)	Fully described NTP study.
	Mouse; M, F 120	Drinking water	0, 0.5, 1 or 2 %, equivalent to 0, 540, 1040 or 2070 mg/kg bw/day in males and 0, 510, 1020 or 2100 mg/kg bw/day in females	2 years	M: None F: 0.5 %, equivalent to 510 mg/kg bw/day	(Cirvello et al., 1995; NTP, 1995b)	Fully described NTP study. NTP conclusions on carcinogenicity: equivocal evidence of carcinogenic activity in males, some evidence of carcinogenic activity in females.
2-Methylbut-3-en-2-ol [02.123]	Rat; M, F 10	Gavage	0, 30, 150 or 750 mg/kg bw/day 5 days/week	4 weeks	150	(BASF, 1994a)	Full study details provided. Study considered valid. Dosing only 5 days/week

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

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
							but a clear NOAEL.
(Linalool [02.013])	Rat; M, F NR	Diet[2]	0 or 50 mg/kg bw/day[1]	84 days	50[1]	(Oser, 1967)	Unpublished report. Administered as 50 % mixture of linalool and citronellol. Slight retardation of growth in males at 50 mg/kg bw/day, not related to food intake.
	Mouse; F 30	Gavage	0 or 365 mg/kg bw/day	5 days	375[1, 3]	(Gaworski et al., 1994)	Immunotoxicity study only.
	Rat; F 100	Gavage		21 days	500	(Lewis, 2006)	
(Linalyl acetate [09.013])	Rat; M, F NR	Diet[5]	0 or 24 mg/kg bw/day[1]	84 days	24[1]	(Oser, 1967)	Unpublished report. Administered as mixture of linalyl acetate (24 mg/kg bw/day), linalyl isobutyrate (27 mg/kg bw/day) and geranyl acetate (48 mg/kg bw/day). Slight growth retardation of females.
(Linalyl isobutyrate [09.423])	Rat; M, F NR	Diet[6]	0 or 27 mg/kg bw/day[1]	84 days	27[1]	(Oser, 1967)	
	Rat; NR 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw/day	18 weeks	500 (10,000 ppm)	(Hagan et al., 1967)	Very limited details provided.
(Linalyl cinnamate [09.736][7])	Rat; M, F 10	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw/day	17 weeks	500[1] (10,000 ppm)	(Hagan et al., 1967)	Very limited details provided.
(Geranyl acetate [09.011] /Citronellyl acetate[09.012][8])	Mouse; M, F 50	Gavage		103 weeks	-[9]	(NTP, 1987a)	
	Rat; M, F 50	Gavage		103 weeks	1000 (710; 290)[8]	(NTP, 1987a)	
(Myrcene [01.008])	Rat; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw/day	13 weeks	None	(NTP, 2004a)	Draft results only available. Evaluated by the JECFA. No NOAEL as most sensitive adverse effect (nephropathy) present at lowest dose tested (JECFA, 2006a).
	Mouse; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw/day, 5 days/week	13 weeks	Male: None Female: 250 mg/kg bw/day	(NTP, 2004a)	Draft results only available. Evluated by the JECFA. Liver hypertrophy in males at all doses (JECFA, 2006a).
(Menthol [02.015])	Mouse; M, F 50	Diet	0, 2000 or 4000 ppm, equivalent to 0, 300 or 600 mg/kg bw/day	103 weeks	600[1]	(National Cancer Institute, 1979)	Good quality.
	Mouse; F 30	I.P. injection	0, 500 or 2000 mg/kg, 3 times/week	24 weeks	None	(Stoner et al., 1973)	Good quality.
	Rat; M, F 20	Gavage	0, 200, 400 or 800 mg/kg bw day	28 days	None	(Thorup et al., 1983a)	Relative good quality.
	Rat; M, F 80	Diet	0, 100 or 200 mg/kg bw	5.5 weeks	200[1]	(Herken, 1961)	Limited information.
	Rat; M, F 50	Diet	0, 3750 or 7500 ppm, equivalent to 0, 188 or	103 weeks	375[1]	(National Cancer Institute, 1979)	Good quality.

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

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			375 mg/kg bw/day				
(Terpinyl acetate [09.830])	Rat; M, F 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw/day	20 weeks	500[1]	(Hagan et al., 1967)	Very limited details provided.
Sclareol [02.206]	Rat; M, F 20	Gavage	8.8 mg/kg bw/day	28 days	8.8	(IOFI, 2006a)	
Cedrol [02.120]	Rat; M, F 20	Gavage	8.4 mg/kg bw/day	28 days	8.4	(IOFI, 2006a)	
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat; M, F 40	Gavage	10, 50, 500, 1000 mg/kg bw/day	90 days	50 (F) 10 (M)	(Dunster et al., 2007)	
Bisabola-1,12-dien-8-ol [02.129]	Dog, NR 20	Gavage	1 ml/kg bw	14 days	850	(Habersang et al., 1979)	
	Rat, M,F 20	Gavage	2, 3 ml/kg bw	28 days	850	(Habersang et al., 1979)	

NR=Not Reported

[1]*The study was performed at a single dose level or multiple dose levels that produced no adverse effects.*

[2]*Administered with citronellol as part of a 50/50 mixture.*

[3]*Immunotoxicity study.*

[4]*Three treatment groups per sex. Each dose group included 60 male and female animals.*

[5]*Administered with linalyl isobutyrate and geranyl acetate as a part of a mixture.*

[6]*Administered with linalyl acetate and geranyl acetate as a part of a mixture.*

[7] *Structurally related ester of linalool not evaluated as part of the supporting chemicals group.*

[8] *Structurally related terpenoid esters administered as a mixture: geranyl acetate, 71 %; citronellyl acetate, 29 %.*

[9] *A NOEL could not be established due to a high incidence of gavage errors and low survival associated with pneumonia.*

Developmental and reproductive toxicity data are available for two candidate substances of the present Flavouring Group Evaluation from chemical group 6 and 8, and for three of the supporting substances evaluated by the JECFA at the 51st meeting (JECFA, 1999a) or at the 63rd meeting (JECFA, 2006a). The supporting substances are listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Study type Duration	Species/Sex No/group	Route	Dose levels	NOAEL (mg/kg/day) including information on possible maternal toxicity	Reference	Comments
2-Methylpropan-2-ol [02.052]	Developmental Toxicity Gestation Days 6– 20	Mouse; F 15	Liquid Diet	0, 0.5, 0.75 or 1 % (equivalent to approximately 0, 3400, 4900 and 6400 mg/kg bw/day)	Maternal: 0.5 % Foetal: None	(Daniel & Evans, 1982)	Study considered valid. Dose related reduction of offspring performance in neurobehavioural tests (righting reflex, negative geotaxis, open file behaviour, cliff avoidance, roto-rod performance).
	Developmental Toxicity: Gestation Days 6– 18	Mouse; F 9-12	Gavage[1]	0 or 10.5 mmol/kg bw/day, equivalent to 1557 mg/kg bw/day	Maternal: None Foetal: None	(Faulkner et al., 1989)	Study considered valid. Increased foetal resorptions and decreased births per litter in treated group.
(Linalool [02.013])	Reproductive & Developmental Toxicity: 33 – 39 days[3]	Rat; F 10	Gavage	0, 250, 500 or 1000 mg/kg bw/day coriander oil, equivalent to 0, 182.3, 364.5 or 729 mg/kg bw/day linalool	Maternal: None Foetal: 364.5	(Hoberman & Christian, 1989)	Test substance was coriander oil. Linalool content 72.9 %.
(Menthol [02.015])	Teratology Gestation days 6-15	Mouse; F 22	Gavage	0, 1.85, 8.59, 39.9, 185	185[2]	(Food and Drug Research Laboratories, Inc., 1973)	
	Teratology Gestation days 6-15	Rat; F 22-23	Gavage	0, 2.18, 10.15, 47.05, 218	218[2]	(Food and Drug Research Laboratories, Inc., 1973)	
	Teratology Gestation days 6-15	Hamster; F 20-22	Gavage	0, 4.05, 21.15, 98.2, 405	405[2]	(Food and Drug Research Laboratories, Inc., 1973)	
	Teratology Gestation days 6-18	Rabbit; F 9-11	Gavage	0, 4.25, 19.75, 91.7, 425	425[2]	(Food and Drug Research Laboratories, Inc., 1973)	
(Myrcene [01.008])	Teratology Gestation days 6-15	Rat; F 16-29	Gavage	0, 250, 500, 1200 mg/kg bw/day	Maternal: 500 mg/kg bw/day Foetal: 500 mg/kg bw/day	(Delgado et al., 1993a)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Gestation day 15 to postanatal day 21	Rat; F 12-18	Gavage	0, 250, 500, 1000, 1500 mg/kg bw/day	Maternal: 500 mg/kg bw/day Foetal/neonatal: 250 mg/kg bw/day	(Delgado et al., 1993b)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Prior to mating until postnatal day 21[4]	Rat; M, F 60	Gavage	0, 100, 300, 500 mg/kg bw/day	Maternal/paternal: 500 mg/kg bw/day Foetal: 300 mg/kg bw/day	(Paumgartten et al., 1998)	Study considered valid. Evaluated by the JECFA.
2,6-Dimethyloct-7-en-2-ol	Developmental Toxicity:	Rat, M, F	Gavage	0, 250, 500, 1000 mg/kg	Maternal: 500 mg/kg	(Politano et al.,	

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Study type Duration	Species/Sex No/group	Route	Dose levels	NOAEL (mg/kg/day) including information on possible maternal toxicity	Reference	Comments
[02.144]	Gestation Days 7– 17	25		bw/day	bw/day Foetal: 500 mg/kg bw/day	2008)	

[1]*Test substance was administered to two strains of mice at a dose level of 778 mg/kg (10.5 mmoles/kg) twice daily.*

[2]*The study was performed at a single dose level or multiple dose levels that produced no adverse effects.*

[3]*Animals were dosed for seven days prior to mating, during mating (maximum of seven days), during gestation, delivery and four days post parturition.*

[4]*Males were dosed for 91 days prior to mating and during mating; females were dosed from 21 days prior to mating to 21 days after birth.*

In vitro mutagenicity/genotoxicity data are available for five candidate substances of the present Flavouring Group Evaluation from chemical group 6 and 8, and for eight supporting substances evaluated by the JECFA at the 51st meeting (JECFA, 1999a) or at the 63rd meeting (JECFA, 2006a). Supporting substances are listed in brackets.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	0.1, 0.5, 2.5, 5, 10 µl/plate (7800 µg/plate)	Questionable[1]	(Haworth et al., 1981a)	Unpublished GLP study. According to the conclusion of the report, the test substance "did cause a weak but significant increase in TA1535 revertants per plate in both the presence and absence of rat liver microsomes." However, this result cannot be re-evaluated because the corresponding page with results on TA1535 in Table format is lacking.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	probably 1 to 10 µl/plate (7800 µg/plate) (corresponding pages of the report are lacking)	Questionable[1]	(Haworth et al., 1981b)	Unpublished GLP study. According to the conclusion of the report, the test substance (purity 99.9 %) "did not cause a significant increase in the number of revertants per plate in any of the tester strains with or without metabolic activation. It should be noted, however, that there was a slight increase in TA1535 revertants per plate observed in the presence and absence of rat liver microsomes." However, this result cannot be re-evaluated because 15 pages with all results in Table format are lacking.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	10000 µg/plate	Negative[1]	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD guideline 471. There was a slight increase in TA1535 revertants per plate observed in the presence and absence of rat and hamster liver microsomes. This effect is dose-related only with hamster liver S9. Overall, the effects were less than twice compared to control. The study is considered valid.
	Yeast mitochondrial mutation assay	Several *Saccharomyces strains*	4 %	Positive	(Jiménez et al., 1988)	This non-GLP study was not in accordance with OECD guideline 480 (1986), and the study protocol does not belong to standard protocols used in routine testing. However, the result is considered valid since main details of method and results are reported. Endpoint not relevant for genotoxicity.
	Forward mutation assay	Mouse lymphoma L5178Y TK +/-	0, 1000, 2000, 3000, 4000, 5000 µg/ml	Negative[1]	(McGregor et al., 1988b)	Non-GLP study in accordance with OECD guideline 476 (1984). Study is considered valid.
	Forward mutation assay	Mouse lymphoma L5178Y TK +/-	1.3 to 100 µl/ml (78000 µg/ml)	Negative	(Kirby et al., 1981)	Unpublished GLP study. According to the report's summary, test substance of high (99.9 %) purity did not induce any detectable increases in the mutant frequencies in the presence and absence of S9-mix. When cultures were tested in the presence of S9-mix with less pure test substance none of the cultures exhibited increases in mutant frequency. Without S9-mix this test substance did appear to induce an increase in the mutant frequency of cultures treated with the higher doses, but a dose-related response was not evident. In addition, in only one of two experiments was a greater than two-fold increase in mutant frequency observed. However, this result cannot be re-evaluated because 47 pages with all results in Table format are lacking in the report submitted.
	Chromosomal aberration	Chinese hamster ovary cells	160 to 5000 microgram/ml	Negative	(NTP, 1984e)	Limited validity. This non-GLP study was in accordance with OECD guideline 473 (1983) except that only a single harvest time

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						was used, however, the study protocol does not fully meet the criteria of the revised guideline from 1997. According to the version from 1997, a single sampling time should be equivalent to about 1.5 normal cell cycle lengths, duplicate cultures should be used at each concentration and 200 metaphases should be scored per concentration.
	Sister chromatid exchange	Chinese hamster ovary cells	6 concentrations ranging from 0.625 to 20 µl/ml (15600 µg/ml)	Negative	(Putman, 1985)	Unpublished GLP study. According to the report's summary, test substance of high (99.9 %) purity caused a significant increase in sister chromatid exchanges at the high dose only in the assay without S9 and at the two highest doses in the assay with S9, however, the test article did not meet the criteria for a positive response (at least two-fold increase or a significant positive dose response over at least three doses). However, this result cannot be re-evaluated because pages with all results in Table format are lacking in the report submitted.
	Sister chromatid exchange	Chinese hamster ovary cells	20 µl/ml (15600 µg/ml)	Negative	(Thilagar et al., 1981)	Unpublished GLP study is considered valid. A marginal increase in SCE frequency was observed in the tests with and without S9, while only the highest concentration without S9 resulted in a significant increase. Thus, the test article did not meet the criteria for a positive response (at least two-fold increase or a significant positive dose response over at least three doses). (All relevant tables were submitted).
2-Methylbutan-2-ol [02.041]	Mutagenicity assays	*S. typhimurium* TA1535; TA1537; TA1538; *S. cerevisiae*	NR	Negative	(Dow Chemical Company, 1982b)	Very short abstract only.
	Sister chromatid exchange	Chinese hamster ovary cells	160, 500, 1600, 5000 µg/ml	Positive[3] Negative[2]	(NTP, 1997c)	
	Sister chromatid exchange	Chinese hamster ovary cells	2000, 3000, 4000, 5000 µg/ml	Negative[1]	(NTP, 1997c)	
2-Methylbut-3-en-2-ol [02.123]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	20 – 5000 µg/plate	Negative[1]	(BASF, 1989d)	Summary in IUCLID data set only. According to this summary, the assay was not in compliance with GLP but in accordance with OECD guideline 471. The unpublished study report is not available for re-evaluation.
	Liquid suspension assay	*S. typhimurium* TA98; TA100	20 – 5000 µg/plate	Negative[1]	(BASF, 1991a)	Summary in IUCLID data set only. According to this summary, the assay was not in compliance with GLP but in accordance with OECD guideline 471. The unpublished study report is not available for re-evaluation.
Isophytol [02.168]	Ames test	*S. typhimurium* TA97; TA98; TA100; TA1535	100, 333, 1000, 3333, 10000 microgram/plate	Equivocal[1]	(NTP, 1994b)	This non-GLP study is considered valid. It is in accordance with OECD guideline 471 (1983). The study is published in the Web and the report contains sufficient details.
	Ames test	*S. typhimurium* TA97, TA98, TA100, and TA1535	five doses from 100 to 10000 microgram/plate	Negative	(NTP, 2000c)	This non-GLP study is considered valid. It is in accordance with OECD guideline 471 (1983). The study is published in the Web and the report contains sufficient details.
(Linalool [02.013])	Ames test (modified)	*S. typhimurium*	3 µl/2 ml	Negative[1]	(Eder et al., 1980)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA100	(2610 µg/2ml) incubation volume 1 mg/plate			
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537	(1000 µg/plate)	Negative[1]	(Ishidate et al., 1984)	
	Ames test	*S. typhimurium* TA98; TA100	100 µl (87000 µg)	Negative[1]	(Rockwell & Raw, 1979)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10000 nl/plate (8700 µg/plate)	Negative[1]	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster fibroblasts	0.25 mg/ml (250 µg/ml)	Negative[1]	(Ishidate et al., 1984)	
	Unscheduled DNA synthesis	Rat hepatocytes	50 nl/ml (43.6 µg/ml)	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Mutation assay	*E. coli* WP2 *uvrA*	1 mg/plate (1000 µg/plate)	Negative	(Yoo, 1986)	In Japanese (only summary and tables in English). Thus, the validity cannot be evaluated.
	Rec assay	*B. subtilis* H17 (rec+); M45 (rec-)	17 µg	Negative	(Oda et al., 1979)	
	Rec assay	*B. subtilis* H17 (rec+); M45 (rec-)	10 µl/disk (8700 µg/disk)	Positive	(Yoo, 1986)	In Japanese (only summary and tables in English). Thus, the validity cannot be evaluated.
	Mammalian cell mutation	Mouse lymphoma L5178Y TK+/-	3.9 to 300 nl/ml	Negative[2] Positive[3]	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	1000 µM (154250 µg)	Negative[1, 4]	(Sasaki et al., 1989)	
(Linalyl acetate [09.013])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	25000 nl/plate (22575µg/plate)	Negative[1]	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Unscheduled DNA synthesis	Fischer or SD rat hepatocytes	300 nl/ml (271 µg/ml)	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Rec assay	*B. subtilis* H17 (rec+); M45 (rec-)	18 µg	Negative	(Oda et al., 1979)	
	Chromosome aberration	Peripheral human lymphocytes	180 µg/ml	Negative[1]	(Bertens & van de Waart, 2000)	
(alpha-Terpineol [02.014])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10000 µg/plate	Negative	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Ames test	*S. typhimurium* TA97a; TA98; TA100;	2500 µg/plate	Negative	(Gomes-Carneiro et al., 1998)	The study is concidered valid. A slight but dose-related response was noted with TA102 with and without the use of metabolic activation.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA102	2500 µg/plate	Weakly positive[5]		
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate	Negative[1]	(National Cancer Institute, 1983)	
	Ames test	*S. typhimurium* TA98 ; TA100 ; TA1535 ; TA1537 ; TA1538	10000 µg/plate	Negative[1]	(Lorillard, 1983b)	
	Spot test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µ mol/plate (463 µg/plate)	Negative[1]	(Florin et al., 1980)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	0.5 µl/ml (467µg/ml) 0.75µl/ml (700 µg/ml)	Negative[3] Negative[2]	(Kirby et al., 1984)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	300 nl/ml (280 µg/ml) 250 nl/ml (233 µg/ml)	Negative[1]	(Heck et al., 1989)	Some important details of method and results are not reported. Thus, the validity of this study cannot be evaluated.
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	15.6 -250 nl/ml 15.6 -300 nl/ml	Negative[2] Negative[3]	(Lorillard, 1982)	
	Rec assay	*S. cerevisiae*	NR	Negative	(Oda et al., 1979)	
(Terpinyl acetate [09.830])	Rec assay	*B. subtilis* H17; M45	19 µg	Negative	(Oda et al., 1979)	
(beta-Terpineol) [02.097])	Ames	*S. typhimurium* TA98; TA100	0.05 µl (100 µl)	Negative[1]	(Rockwell & Raw, 1979)	
	Rec assay	*S. cerevisiae*	NR	Negative[1]	(Oda et al., 1979)	Article does not specify alpha- or beta-terpineol.
(1-Isopropyl-4-methylbenzene [01.002])	*In vivo/in vitro* Ames test	*S. typhimurium* TA98 and TA100	0.5 ml (equivalent to 1,706 mg/kg bw) administered to Sprague-Dawley rats, urine collected and tested *in vitro*	Negative[5]	(Rockwell & Raw, 1979)	
(Myrcene [01.008])	Ames test	*S. typhimurium* TA100; TA1535; TA97; TA98	0, 33, 100, 333, 1000, 3333 and 10000 µg/plate	Negative[1]	(NTP, 1999b)	
	Chromosome aberration	Human lymphocytes	0, 100, 500 and 1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	
	Sister chromatid exchange	Human lymphocytes	0, 100, 500 and 1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	
	HPRT assay	V79 Chinese hamster cells	0, 100, 500 and 1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	
	Sister chromatid exchange	V79 Chinese hamster cells	0, 100, 250 and 500 µg/ml	Negative[1]	(Röscheisen et al., 1991)	
	Sister chromatid exchange	HTC cells	0, 100, 250 and 500 µg/ml	Negative	(Röscheisen et al., 1991)	
(Menthol [02.015])	Ames test	*S. typhimurium*, TA92, TA100, TA94, TA98,	0, and 6 concentrations up to 5000 µg/plate	Negative[1]	(Ishidate et al., 1984)	d,l-Menthol was used. The study is considered valid.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA1535, TA1537				
	Ames test (preincubation method)	*S. typhimurium,* TA1535, TA97, TA100, TA98	3 – 666 µg/plate	Negative[1]	(Zeiger et al., 1988)	d,l-Menthol was used. The study is considered valid.
	Ames test	*S. typhimurium,* TA2637, TA100, TA98	0, 5 – 500 µg/plate	Negative[1]	(Nohmi et al., 1985)	d,l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium,* TA2637, TA100, TA98	0, 20 – 500 µg/plate	Negative[1]	(Nohmi et al., 1985)	l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium,* TA1537, TA1535, TA100, TA98	0, 6.4, 32, 160, and 800 µg/plate	Negative[1]	(Andersen & Jensen, 1984b)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.
	Ames test	*E. coli WP2 uvrA* (Trp^-)	100 – 800 µg/plate	Negative	(Yoo, 1986)	l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.
	Ames test	*S. typhimurium* TA97A; TA98; TA100; TA102	0, 5 – 800 µg/plate	Negative[1]	(Gomes-Carneiro et al., 1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.
	Rec assay	*B. subtilis* H17, M45	Up to 10000 µg/disk	Positive	(Yoo, 1986)	l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method (Rec-assay) has poor predictive value.
	Rec assay	*B. subtilis* H17, M45	20 µg/disk	Negative	(Oda et al., 1979)	l-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (Rec-assay) has poor predictive value.
	Alkaline elution assay	Rat hepatocytes	0, 0.1 – 1.3 mM (203.2 µg/ml[4])	Negative	(Storer et al., 1996)	The experiment employed d-Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.
	Sister chromatid exchange	Chinese hamster ovary cells	5 – 50 amd 0, 2 – 25 µg/ml[3] 0, 16 – 167 µg/ml [2]	Negative[1]	(Ivett et al., 1989)	d,l-Menthol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/ml[4])	Negative[1]	(Murthy et al., 1991)	The study is considered valid.
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1, 10 µg/ml	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 and three concentrations up to 200 µg/ml	Negative[3]	(Ishidate et al., 1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.
	Chromosome aberration	Chinese hamster ovary cells	0, 50 – 250 µg/ml	Negative[1]	(Ivett et al., 1989)	d,l-Menthol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Chromosome	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/ml[4])	Negative[1]	(Murthy et al., 1991)	The study is considered valid.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	aberration					
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0, 12.5 – 200 µg/ml	Negative[1]	(Myhr & Caspary, 1991)	d,l-Menthol was used. The maximum concentration was selected by a preliminary test The study is considered valid.
Bisabola-1,12-dien-8-ol [02.129]	Ames test	*S. typhimurium,* TA98, TA100, TA102, TA1535, TA1537	0, 0.5 – 1500 µg/plate	Negative[1]	(King & Harnasch, 2002b)	
	Ames test	*S. typhimurium,* TA97a, TA98, TA100, TA1535	0, 1-400 µg/plate in ethanol	Negative[1]	(Gomes-Carneiro et al., 2005b)	Publication in peer-reviewed journal. No reference to OECD and GLP-guidelines being made, but study is of good quality and considered valid.
	Ames test	*S. typhimurium,* TA98, TA100, TA1535, TA1537	0, 20-5000 µg/plate in DMSO	Negative[1]	(CIR, 1999)	Review.
	Chromosome aberration	Chinese hamster fibroblast cells V79	0, 0.78-40 µg/ml in DMSO	Negative[1]	(CIR, 1999)	Review.
Cedrol [02.120]	Ames test	*S. typhimurium,* TA97a, TA98, TA102, TA1535	0 – 5000 µg/plate	Negative[1]	(Scheerbaum, 2001)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.
1,2-Dihydrolinalool [02.140]	Ames test	*S. typhimurium,* TA97, TA98, TA100, TA102, TA1535	0 – 1000 µg/plate	Negative[1]	(Gocke, 1999a)	Research report according to OECD and GLP-guidelines, the study is considered valid.
2,6-Dimethyloct-7-en-2-ol [02.144]	Ames test	*S. typhimurium,*TA98, TA100, TA102, TA1535, TA1537	0 – 5000 µg/plate	Negative[1]	(King, 2000)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.
1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol [02.197]	Ames test	*S. typhimurium,*TA98, TA100, TA1535, TA1537 *E. coli WP2 uvrA*	0 – 250 µg/plate[3] 0 – 500 µg/plate[2]	Negative	(Watanabe, 2002)	Research report according to OECD guideline 471, the study is considered valid.

NR = Not Reported.

[1]*With and without metabolic activation.*

[2]*With metabolic activation.*

[3] *Without metabolic activation.*

[4] *With and without pre-treatment with mitomycin C at 0.15 microM for 21 hours.*

[5]*With and without presence of beta-glucuronidase.*

In vivo mutagenicity/genotoxicity data are available for two of the candidate substances of the present Flavouring Group Evaluation from chemical group 6 and 8, and for three supporting substances evaluated by the JECFA at the 51st meeting (JECFA, 1999a) or at the 63rd meeting (JECFA, 2006a). Supporting substances are listed in brackets.

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

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	*In vivo* Chromosomal Aberration assay	Male rats	Once via gavage	0.2xLD50	Positive[1]	(Barilyak & Kozachuk, 1988)	Validity questionable. This study was not in compliance with GLP and not in accordance with OECD guideline No. 475 (1983). Some main details of method and results are not available. The authors report the results of tests on a series of monohydric alcohols (from C1 to C16, 18 compounds) in rat bone marrow cytogenetic tests. All compounds were claimed positive compared to the untreated control group, even though no statistics is shown. It is noted that a single control group, with 0.0 % of cells with aberrations was used throughout the study. Lacking historical control data, it is not possible to establish whether the alleged positive results were due to and uniformly positive response elicited by all chemicals, or rather by an incidental very low frequency of aberrations in the group of rats (8 animals) used as control. In this respect it is noted that the incidence of chromosomal aberrations observed with some "positive" compounds, including 2-methylpropan-2-ol (1.6+/-0.5 %), are close to background incidences commonly observed. Even the lack of a concurrent raise in gaps in treated animals casts doubts on an induced genotoxic effect. Moreover, the lack of a positive control group in the study is noted. For these reasons, the results of this study are considered inconclusive.
	In vivo Micronucleus assay	Mouse bone marrow erythrocytes	I.P. x 3 at 24 hours intervals (=72 hours)	312.5, 625, and 1250 mg/kg bw	Negative	(NTP, 1996c)	This study is considered valid. It was not in compliance with GLP but in accordance with OECD guideline No. 474 (1983/1997) except that only 5 male animals were tested. The study is published in the Web and the report contains sufficient details. Due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance has reached the bone marrow. Relevance of the result is limited.
	In vivo Micronucleus assay	Rat bone marrow cells	I.P. x 3 at 24 hours intervals (=72 h)	0, 39 – 1250 mg/kg bw,	Negative	(NTP, 1997c)	
	In vivo Micronucleus assay	Mouse peripheral blood cells	Drinking water	3000 - 40000	Negative	(NTP, 1995b)	
2-Methylbut-3-en-2-ol [02.123]	*In vivo* Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	500, 1000, 1500 mg/kg	Negative	(BASF, 1992b)	Summary in IUCLID data set only. According to this summary, the assay was perfomed in compliance with GLP and in accordance with OECD guideline 474. One thousand PCEs were conted per animal. The unpublished study report is not available for re-evaluation.
(Linalool [02.013])	*In vivo* Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	1500 mg/kg	Negative	(Meerts & van de Waart, 2001)	This study is considered valid. It was in compliance with GLP and in accordance with OECD Guideline 474 (1997). However, due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance reached the bone marrow. Thus, the relevance of the result is limited.
(Myrcene [01.008])	*In vivo* Micronucleus assay	Rat bone marrow cells	Gavage	0, 100, 500 or 1000 mg/kg bw	Negative	(Zamith et al., 1993)	
	In vivo Micronucleus assay	Mouse peripheral blood cells	Gavage	Up to 2000 mg/kg bw/day for 13 weeks	Negative	(NTP, 2001b)	
(Menthol [02.015])	Host mediated	*S. typhimurium*	Gavage	0, 1.45 - 5000 mg/kg	Equivocal	(Food and Drug	Negative results, with exception of the combination *S. typhimurium*

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

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
	mutation assay	TA1530 and G46; *S. cerevisiae* D3 inoculated in mice (7-9 animals/group)		bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)		Research Laboratories, Inc., 1975a)	TA1530 – 5000 mg/kg bw and *S. cerevisiae* D3 – 1150 mg/kg bw/day. This study is considered valid, but the equivocalresult might have low relevance since the effect was only observed at very high (lethal) dose levels.
	In vivo Cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	Oral DL_{50} was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.
	In vivo Micronucleus assay	B6C3F1 male mouse bone marrow cells	I.P.	0, 250 - 1000 mg/kg bw/day, during 3 days	Negative	(Shelby et al., 1993)	d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	In vivo Dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	This study is considered valid.

[1]*Cytogenetic analysis indicated the following results for controls and 2-methylpropan-2-ol, respectively: % polyploid cells, 0.5 ± 0.3 / 0.8 ± 0.4; % cells with gaps 0.3 ± 0.2 / 0.4 ± 0.2; % cells with aberrations, 0 / 1.6 ± 0.5. Statistical comparisons were not performed.*

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
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)
GLP	Good Laboratory Practice
ID	Identity
IOFI	International Organization of the Flavour Industry
IP	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NMR	Nuclear Magnetic Resonance
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food

SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation