

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3): 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}

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 29 flavouring substances in the Flavouring Group Evaluation 18, as laid down in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have a 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. This revision deals with new toxicity data on the supporting substance myrcene [FL-no: 01.008], providing an appropriate NOAEL for the evaluation of candidate substance [FL-no: 02.146]. The Panel concluded that all 29 substances [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.129, 02.140, 02.146, 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. However, based on mTAMDI calculations, for all candidate substances except [FL-no: 02.146] more reliable intake data are required for a re-evaluation. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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KEY WORDS

flavourings, safety, aliphatic, alicyclic, saturated, unsaturated, tertiary alcohols, FGE.18

¹ On request from the European Commission, Question No EFSA-Q-2013-00852, adopted on 07 May 2015.

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to deliver a scientific opinion 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 29 flavouring substances in the Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 29 flavouring substances belong to chemical groups 6 and 8, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision 3 of FGE.18 (FGE.18Rev3) is due to the availability of new toxicity data for myrcene, [FL-no: 01.008] which was used for the safety evaluation of the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

FGE.18Rev3 deals with 29 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.

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four substances [FL-nos: 02.129, 02.147, 02.168 and 02.197] the stereoisomeric composition of the mixture has not been specified.

Seventeen of the 29 candidate substances are classified into structural class I, eleven into structural class II and one into structural class III according to the decision tree approach.

Twenty-one out of the 29 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.

According to the default MSDI approach, 28 of the 29 flavouring substances in this group have intakes in Europe that vary from 0.0012 to 27 $\mu\text{g}/\text{capita}$ per day, which are below the thresholds of concern for structural classes I, II and III substance of 1800, 540 and 90 μg per person per day, respectively. The remaining substance, terpineol [FL-no: 02.230] belonging to structural class I, has an MSDI of 1200 $\mu\text{g}/\text{capita}$ per day, which is also below the threshold for this structural class.

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 candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no metabolism data are available and therefore it cannot be predicted to be metabolised to innocuous products.

In Revision 2 of FGE.18, for substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no appropriate NOAEL was available and additional data were requested. In the present revision of FGE.18 (Revision 3), new toxicity data on the supporting substance myrcene [FL-no: 01.008] became available, providing an appropriate NOAEL of 44 mg/kg bw per day which was used for the evaluation of the candidate substance [FL-no: 02.146]. Based on the MSDI intake of 12 µg/capita per day, the margin of safety calculated for [FL-no: 02.146] is 2.2×10^5 .

Therefore, none of the 29 candidate substances [FL-nos: 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 give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances, on the basis of the default MSDI approach.

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 25 candidate substances. However, information on purity criteria and/or stereoisomeric composition has not been specified sufficiently for four substances [FL-no: 02.129, 02.147, 02.168 and 02.197]. Thus, the final evaluation of the materials of commerce cannot be performed for these substances, pending further information.

Thus, for 25 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach: [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.146, 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].

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI approach, are 3900 µg per person per day, and for the two remaining substances 7000 and 14000 µg per person per day respectively. These seventeen mTAMDI are all above the threshold of concern of 1800 µg per person per day for a structural class I substance. For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg per person per day and for the remaining two substances, 110 and 1600 µg per person per day, respectively. For ten substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg per person per day. For the one substance assigned to structural class III the mTAMDI is 5700 µg per person per day, which is above the threshold of concern for structural class III substances of 90 µg per person per day.

In conclusion, for all candidate substances except [FL-no: 02.146] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008⁴ on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012⁵. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000.⁶

FGE.78Rev1

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2.⁷

The substances [FL-nos: 01.008, 01.018, 01.040 and 01.061] were among the 14 substances for which the Panel had “reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing information on stereoisomerism/composition of mixture)” and also among those for which “additional toxicity data was requested”.

FGE.25Rev2

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31.⁸

The substances with [FL-nos: 01.035, 01.064, 01.070 and 01.035] were among the 27 candidate substances for which “additional toxicity data” were required by EFSA. For [FL-no: 01.035] also “additional information on composition” was requested.

FGE.18Rev2

On 30 September 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an 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.

For the flavouring substance [FL-no: 02.146], the Panel considered that “additional data” are needed including “information on specifications/stereoisomerism/composition of mixture”.

⁴ Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p.34-50.

⁵ Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1-161.

⁶ Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8-16.

⁷ EFSA Journal 2011;9(6):2178

⁸ EFSA Journal 2011;9(6):2177

On 21 November 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted a statement on the re-evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] based on additional data on a supporting substance.⁹

The Panel concluded that “linalool [FL-no: 02.013] is not sufficiently structurally related to 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for a re-evaluation of [FL-no: 02.146]. Accordingly, a 90-day study on 3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] or on a sufficiently structurally related substance has to be provided in order to establish on appropriate NOAEL”.

New data and relationship with other substances

On 5 and 11 July 2013, the applicant submitted additional data on the following acyclic terpene hydrocarbons [FL-nos: 01.008, 01.018, 01.040, 01.061, 01.035, 01.064, 01.070 and 02.146] represented by myrcene [FL-no: 01.008].

As regards the related substances also evaluated in these opinions, namely [FL-nos: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026, 01.029 and 01.059], data was submitted and are currently being evaluated (EFSA-Q-2013-00185 to – 00193).

As regards substance with [FL-no: 01.014], data should be submitted by 31 December 2013.¹⁰

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment on this group of flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

SUPPORTING DOCUMENTS

Submission by the European Flavour Association

INTERPRETATION OF THE TERMS OF REFERENCE

The above background and terms of reference include also a previous mandate received from the European Commission on 6 February 2013.¹¹ The present scientific opinion FGE.18Rev3 covers the safety assessment of the following flavouring substance: 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

⁹ EFSA Journal 2012;10(12):2995

¹⁰ This substance is in the process of being deleted from the Union List (DG SANTE, 2015)

¹¹ SANCO.E3/SH/km D (2013) Ares(2013)15188

ASSESSMENT

1. History of the Evaluation of the Substances in the Present FGE

The first version of the Flavouring Group Evaluation 18, FGE.18, dealt with 24 aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters.

The first Revision of FGE.18, FGE.18Rev1, included the assessment of six additional candidate substances [FL-nos: 02.120, 02.206, 02.226, 02.230, 02.253 and 09171]. Information on toxicity and/or metabolism on three of the substances were included [FL-nos: 02.120, 02.206 and 02.230]. Since the publication of FGE.18 additional information on specifications on 12 substances became available [FL-nos: 02.052, 02.140, 02.144, 02.147, 02.149, 02.150, 02.168, 02.191, 02.197, 09.614, 09.617 and 09.671].

The second Revision of FGE.18, FGE.18Rev2, included the assessment of two additional candidate substances [FL-nos: 02.129 and 02.146]. Information on toxicity and/or metabolism on the substance [FL-no: 02.129] was included. No information on toxicity and/or metabolism was available for [FL-no: 02.146]. A search in open literature did not provide any further data on toxicity or metabolism for these substances. Since the publication of FGE.18Rev1 additional information on toxicity on [FL-nos: 02.120, 02.140, 02.144 and 02.197] was included. Furthermore, additional information on specifications on six substances was included [FL-nos: 02.147, 02.168, 02.197, 02.226, 02.230 and 02.253] (EFFA, 2010). Based on the additional information made available by Industry, it was also considered appropriate to merge subgroups 7 and 8 into one (subgroup 7).

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	http://www.efsa.europa.eu/en/efsajournal/pub/1847.htm	32
FGE.18Rev3	7 May 2015	http://www.efsa.europa.eu/en/efsajournal/pub/4118.htm	29

The present Revision of FGE.18, FGE.18Rev3, includes the safety assessment of (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for which a 90-day toxicity study on a supporting substance has been provided. No data on toxicity and/or metabolism are available for the substance itself and a search in the open literature did not provide any pertinent information either. In the EFSA statement of 2012 (EFSA, 2012) the Panel has proposed a suitable supporting substance for [FL-no: 02.146], namely myrcene [FL-no: 01.008]. The safety evaluation of myrcene has been finalised in FGE.78Rev2 based on a new 90-day toxicity study (Bauter, 2013) for this substance. These new data will accordingly be used in the safety evaluation of (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146]. Substances in subgroup 3 with [FL-nos: 02.185, 02.191 and 09.669] are no longer used as flavouring substances in the EU according to the Industry (DG SANCO, 2012) and therefore they will not be considered in the present FGE.

1.1. Description

The present Flavouring Group Evaluation 18, Revision 3 (FGE.18Rev3) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000) (The Procedure – shown in schematic form in Appendix B of this FGE), deals with 29 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, 2000). The 29 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 Manufacturers 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-nos: 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-nos: 02.123, 02.144, 02.150, 02.168, 02.226, 09.614 and 09.671]; one aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond [FL-no: 02.146]; 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-nos: 02.054, 02.171 and 09.617]; two monocyclic unsaturated tertiary alcohols [FL-nos: 02.129 and 02.230]; two mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond [FL-nos: 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-nos: 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 Appendix A, Table 7.

The hydrolysis products of the candidate esters are listed in Appendix A, Table 8.

The 29 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, 2005b), 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 Appendix A, Table 9, together with their evaluation status.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the FGE.18Rev3



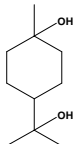
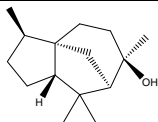

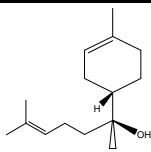
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.041	2-Methylbutan-2-ol		515 75-85-4	Liquid C ₅ H ₁₂ O 88.15	Slightly soluble Freely soluble	102 MS 96 %	1.402-1.408 0.805-0.813	
02.052	2-Methylpropan-2-ol		698 75-65-0	Liquid C ₄ H ₁₀ O 74.12	Slightly soluble Freely soluble	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 Freely soluble	260 116 MS 95 %	n.a. n.a.	
02.120 2030	(+)-Cedrol		4503 10190 77-53-2	Solid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Freely soluble	286 86 MS 95 %	n.a. n.a.	
02.123	2-Methylbut-3-en-2-ol		11794 115-18-4	Liquid C ₅ H ₁₀ O 86.13	Practically insoluble or insoluble Freely soluble	97 MS 98 %	1.413-1.419 0.818-0.827	
02.129 2031	(l)-alpha-Bisabolol	 (l)-alpha-Bisabolol shown	4666 10178 23089-26-1	Liquid C ₁₅ H ₂₆ O 222.37	Insoluble Soluble	^(g) MS >94 %	1.493-1.499 0.927-0.935	Secondary components (no amount given): (d)-alpha- Bisabolol (CAS No. 23178-88-3), (l)-epi-alpha-

Table 1: Specification Summary of the Substances in the FGE.18Rev3

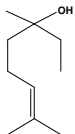
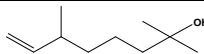
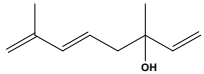
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
								Bisabolol (CAS No.78148-59-1), (d)-epi-alpha-Bisabolol (CAS No. 76738-75-5). Stereoisomeric composition to be specified.
02.140	1,2-Dihydrolinalool		2270-57-7	Liquid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	90 (16 hPa) MS 95 %	1.452-1.458 0.857-0.863	Racemate.
02.144	2,6-Dimethyloct-7-en-2-ol		18479-58-8	Liquid C ₁₀ H ₂₀ O 156.27	Practically insoluble or insoluble Freely soluble	86 (15 hPa) MS 95 %	1.434-1.440 0.824-0.830	Racemate.
02.146	(E)-3,7-Dimethylocta-1,5,7-trien-3-ol ^(f)		10202 53834-70-1	Liquid C ₁₀ H ₁₆ O 152.24	Insoluble	62 (6 hPa) MS 93 %	1.489-1.495 0.878-0.886	Racemate; Secondary components 2-3 % linalool, 1-2 % linalool oxide and up to 1 % nerol oxide (Flavour Industry, 2012).

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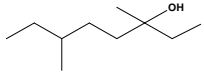
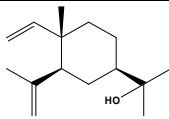
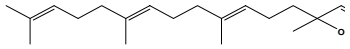
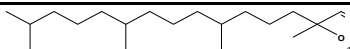
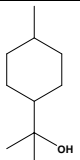
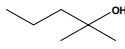
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.147	3,6-Dimethyloctan-3-ol ^(f)		151-19-9	Liquid C ₁₀ H ₂₂ O 158.28	Practically insoluble or insoluble Freely soluble	195 MS 95 %	1.434-1.440 0.831-0.837	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.149	(-)-alpha-Elemol		10205 639-99-6	Solid C ₁₅ H ₂₆ O 222.37	Practically insoluble or insoluble Freely soluble	123 (5 hPa) 50 MS 95 %	n.a. n.a.	Register name (1R,3S,4S)- isomer.
02.150	(E,E)-Geranyl linalool		1113-21-9	Solid C ₂₀ H ₃₄ O 290.49	Practically insoluble or insoluble Freely soluble	153 (5 hPa) 51 MS 95 %	1.484-1.490 0.878-0.881	Racemate of (6E,10E)-isomer.
02.168	Isophytol ^(f)		10233 505-32-8	Liquid C ₂₀ H ₄₀ O 296.54	Practically insoluble or insoluble Freely soluble	138 (0.1 hPa) MS 95 %	1.457-1.462 0.847-0.853	Mixture of diastereo isomers (EFFA, 2010). 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 Freely soluble	208 35 MS 95 %	1.460-1.466 0.909-0.915	
02.181	2-Methylpentan-2-ol		10274 590-36-3	Liquid C ₆ H ₁₄ O 102.18	Slightly soluble Freely soluble	122 MS 95 %	1.409-1.415 0.823-0.829	

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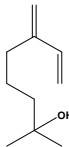
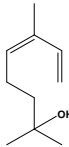
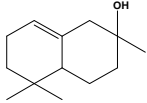
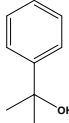
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.184	3-Methylpentan-3-ol		10277 77-74-7	Liquid C ₆ H ₁₄ O 102.18	Practically insoluble or insoluble Freely soluble	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 Freely soluble	91 (13 hPa) MS 95 %	1.470-1.476 0.873-0.879	Not longer supported by industry (DG SANCO, 2012)
02.191	Ocimenol		5986-38-9	Liquid C ₁₀ H ₁₈ O 154.25	Practically insoluble or insoluble Freely soluble	94 (16 hPa) MS 95 %	1.480-1.486 0.871-0.877	Mixture of (E)- and (Z)-isomers (EFFA, 2010). Not longer supported by industry (DG SANCO, 2012)
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol ^(f)		10173 41199-19-3	Solid C ₁₃ H ₂₂ O 194.32	Practically insoluble or insoluble Freely soluble	82 (1 hPa) 68 MS 95 %	n.a. n.a.	Mixture of diastereo isomers (EFFA, 2010). Stereoisomeric composition to be specified.
02.203	2-Phenylpropan-2-ol		11704 617-94-7	Liquid C ₉ H ₁₂ O 136.19	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.529-1.535 0.971-0.977	

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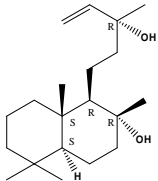
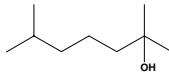
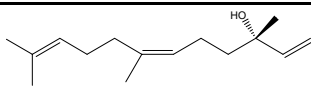
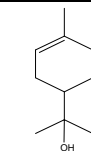
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.206 2029	(-)-Sclareol		4502 10311 515-03-7	Solid $C_{20}H_{36}O_2$ 308.5	Practically insoluble or insoluble Freely soluble	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 Freely soluble	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	
02.230	Terpineol	 alpha-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, 2007b).

Table 1: Specification Summary of the Substances in the FGE.18Rev3

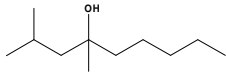
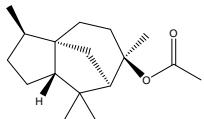
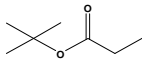
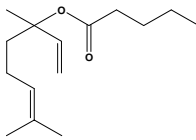
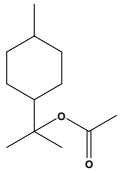
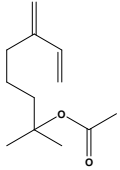
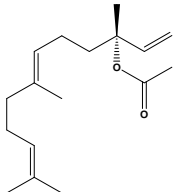
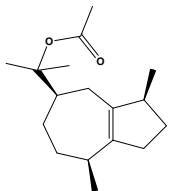
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
02.253 1850	2,4-Dimethyl-4- Nonanol		4407 74356-31-3	Liquid C ₁₁ H ₂₄ O 172.31	Practically insoluble or insoluble Freely soluble	212 MS 95 %	1.434-1.440 0.825-0.833	Racemate (EFFA, 2010).
09.171	Cedryl acetate		527 77-54-3	Solid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	158 (10.7 hPa) 156 MS 95 %	n.a. n.a.	
09.356	1,1-Dimethylethyl propionate		20487-40-5	Liquid C ₇ H ₁₄ O ₂ 130.19	Practically insoluble or insoluble Freely soluble	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 Freely soluble	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 Freely soluble	115 (13 hPa) MS 95 %	1.449-1.455 0.930-0.940	
09.669	Myrcenyl acetate		10857 1118-39-4	Liquid C ₁₂ H ₂₀ O ₂ 196.29	Practically insoluble or insoluble Freely soluble	112 (13 hPa) MS 95 %	1.456-1.462 0.915-0.921	Not longer supported by industry (DG SANCO, 2012)

Table 1: Specification Summary of the Substances in the FGE.18Rev3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility ^(a) Solubility in ethanol ^(b)	Boiling point, °C ^(c) Melting point, °C ID test Assay minimum	Refrac. Index ^(d) Spec.gravity ^(e)	Specification comments
09.671	(3S,6Z)-Nerolidyl acetate		10862 56001-43-5	Liquid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	107 (0.4 hPa) MS 95 %	1.467-1.473 0.901-0.907	
09.808	Guaiyl acetate		10659 134-28-1	Solid C ₁₇ H ₂₈ O ₂ 264.41	Practically insoluble or insoluble Freely soluble	269 96 MS 95 %	n.a. n.a.	

(a): Solubility in water, if not otherwise stated.

(b): Solubility in 95 % ethanol, if not otherwise stated.

(c): At 1013.25 hPa, if not otherwise stated.

(d): At 20°C, if not otherwise stated.

(e): At 25°C, if not otherwise stated.

(f): Stereoisomeric composition not specified.

(g): Boiling point missing.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavours 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.)

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four of these flavouring substances [FL-nos: 02.129, 02.147, 02.168 and 02.197] the stereoisomeric composition has not been sufficiently specified (see Table 1).

1.3. Natural Occurrence in Food

Twenty-one out of 29 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 nine of these 29 substances:

Table 2: Candidate Substances Reported to Occur in Food (TNO, 2000)

FL-no:	Name:	Quantitative data reported
02.041	2-Methylbutan-2-ol	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
02.052	2-Methylpropan-2-ol	0.25 mg/kg in grape, up to 0.1 mg/kg in mango, 0.0021 mg/kg in guava fruit
02.054	p-Menthane-1,8-diol	Up to 0.37 mg/kg in cranberry
02.120	Cedrol	1900 mg/kg in calamus (European), up to 100 mg/kg in cinnamon
02.123	2-Methylbut-3-en-2-ol	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
02.149	Elemol	0.37 mg/kg in grapefruit juice
02.171	p-Menthan-8-ol	0.01 mg/kg in grapefruit juice
02.181	2-Methylpentan-2-ol	0.01 mg/kg in plumcot
02.184	3-Methylpentan-3-ol	0.034 mg/kg in plumcot

According to TNO eight of the substances have not been reported to occur naturally in any food items:

Table 3: Candidate Substances Not Reported to Occur in Food (TNO, 2000)

FL-no:	Name:
02.140	1,2-Dihydrolinalool
02.147	3,6-Dimethyloctan-3-ol
02.150	Geranyl linalool
02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol
02.219	2,6-Dimethyl-2-heptanol
02.253	2,4-Dimethyl-4-nonanol
09.671	Nerolidyl acetate
09.808	Guaiyl acetate

2. Specifications

Purity criteria for the 29 substances have been provided by the Flavouring Industry (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007b; EFFA, 2010; Flavour Industry, 2009a; Flavour Industry, 2009b) (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] are insufficient. Furthermore, the stereoisomeric composition / composition of mixture need to be specified for [FL-nos: 02.129, 02.147, 02.168 and 02.197]. The specifications are adequate for 25 candidate substances (see Section 1.2 and Table 1).

3. Intake Data

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

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

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

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

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake 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, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

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

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), 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 population¹² (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation the total annual volume of production of the 29 candidate substances from use as flavouring substances in Europe has been reported to be approximately 10700 kg (EFFA, 2004b; EFFA, 2005b; EFFA, 2006a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2009b). 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 29 candidate substances, MSDI values for each of these flavourings have been estimated (Appendix A, Table 7).

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 µg/*capita* per day. The daily *per capita* intakes for each of the remaining substances are less than 30 µg (Appendix A, Table 7).

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 29 candidate substances, information on food categories and normal and maximum use levels^{13,14,15} were submitted for the 29 candidate substances by the Flavour Industry (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012). The 29 candidate substances are used in flavoured food products divided into

¹² EU figure 375 million. 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.

¹³ "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

¹⁴ 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).

¹⁵ 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).

the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), as shown in Table 4. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 4: Use of 29 Candidate Substances in Various Food Categories

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

According to the Flavour Industry the normal use levels for the 29 candidate substances are in the range of 0.2 - 200 mg/kg food, and the maximum use levels are in the range of 2 - 500 mg/kg (EFFA, 2002; EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012) (see Appendix C).

The mTAMDI values for the seventeen candidate substances from structural class I (See Section 6) range from 3900 to 14000 µg/person per day. For ten candidate substances from structural class II the

mTAMDI range from 1600 to 3900 µg/person per day whereas for [FL-no: 02.146] from structural class II is 110 µg/person per day. For the one substance from structural class III the mTAMDI value is 5700 µg/person per day (see Section 6).

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

4. Absorption, Distribution, Metabolism and Elimination

Six of the candidate substances in this group are esters [FL-nos: 09.171, 09.356, 09.614, 09.617, 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 5.

Table 5: 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 alcohol with a conjugated terminal double bond
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	

Table 5: Candidate Substances Divided into Subgroups of Related Chemical Structures

Subgroup	FL-no	Candidate substance	Chemical group
6	02.230	Terpineol	Monocyclic and bicyclic unsaturated tertiary alcohols with isolated terminal double bonds
	09.617	p-Menthan-8-yl acetate	
	02.149	Elemol	
	02.206	Sclareol	
7	02.197	1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol	Bi- and tricyclic tertiary alcohols and esters
	02.120	Cedrol	
	09.171	Cedryl acetate	
	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-nos: 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 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 tetrahydrolinalool, mainly conjugated with sulphate or glucuronic acid. The study also indicated that the reduction mainly took place in the gut (Rahman, 1974). 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: The Panel stated that for the evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146], the monoterpene myrcene [FL-no: 01.008] could be used as a supporting substance (EFSA, 2012). This substance also has alcohol moiety 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 substance 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, 1974). *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 glucuronides (Rahman, 1974). 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-nos: 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.

For the candidate substance [FL-no: 02.146], which contains a conjugated terminal double bond, data from a supporting substance, myrcene [FL-no: 01.008] indicate that [FL-no: 02.146] may be oxidised,

giving rise to epoxide intermediates. Thus, it cannot be anticipated that this candidate substance 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 this candidate substance.

A more detailed discussion of the metabolism of the candidate substances in this evaluation is provided in Appendix D.

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 29 candidate substances the Procedure as outlined in Appendix B was applied, based on the MSDI approach. The stepwise evaluations of the 29 substances are summarised in Appendix A, Table 7.

Step 1

Seventeen of the 29 candidate substances are classified into structural class I, eleven candidate substances are classified into structural 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-eight of the candidate substances [FL-nos: 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.

One candidate substance [FL-no: 02.146] contains a conjugated terminal double bond and data from the supporting substance myrcene [FL-no: 01.008] indicate that this terminal double bond 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 Panel decided to evaluate [FL-no: 02.146] according to the Procedure. Since it cannot be anticipated that [FL-no: 02.146] is metabolised to innocuous products, the evaluation of this candidate substance will proceed via the B-side of the Procedure scheme.

Step A3

Seventeen of the candidate substances [FL-nos: 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 µg. These intakes are below the threshold of concern of 1800 µg/person per day for structural class I.

Ten of the candidate substances [FL-nos: 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 µg. These intakes are below the threshold of concern of 540 µg/person per 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 µg. This intake is below the threshold of concern of 90 µg/person per day for structural class III.

Based on results of the safety evaluation sequence these 28 candidate substances [FL-nos: 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 candidate substance [FL-no: 02.146] which could not be predicted to be metabolised to innocuous products,] was assigned to structural class II according to the Cramer classification. The estimated European daily *per capita* intake (MSDI) is of 12 µg for [FL-no: 02.146] is below the threshold of concern of 540 µg per person per day and accordingly, [FL-no: 02.146] proceeds to step B4 of the Procedure.

Step B4

In its statement of 2012 (EFSA CEF Panel, 2012) the Panel identified myrcene [FL-no: 01.008] as supporting substance for [FL-no: 02.146] in the present FGE. The safety evaluation of myrcene has been finalised in FGE.78Rev2 based on a new 90-day toxicity study (Bauter, 2013) which provided a NOAEL of 44 mg/kg bw per day. When the MSDI of [FL-no: 02.146] of 12 µg/*capita* per day is compared to this NOAEL a margin of safety of 2.2×10^5 for [FL-no: 02.146] can be calculated.

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

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI, are 3900 µg/person per day, and for the two remaining substances 7000 and 14000 µg/person per day. These seventeen mTAMDI are all above the threshold of concern of 1800 µg/person per day for a structural class I substance.

For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg/person per day and for the remaining two substances, 110 and 1600 µg/person per day, respectively. For 10 substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg/person per day. For the candidate substance [FL-no: 02.146] the estimated intake is below the threshold of concern for structural class II substances of 540 µg/person per day for a structural class II substance.

For substance [FL-no: 02.129] assigned to structural class III the mTAMDI is 5700 µg/person per day, which is above the threshold of concern for structural class III substances of 90 µg/person per day.

Thus, for 28 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.

Table 6: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI µg/capita per day	mTAMDI µg/person per day	Structural class	Threshold of concern µg/person per 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	(-)-alpha-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	(3S,6Z)-Nerolidyl acetate	0.061	3900	Class I	1800
09.808	Guaiyl acetate	0.0012	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
02.147	3,6-Dimethyloctan-3-ol	0.0012	3900	Class II	540
02.150	(E,E)-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
02.146	(E)-3,7-Dimethylocta- 1,5,7-trien-3-ol	12	110	Class II	540
02.129	(l)-alpha-Bisabolol	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.

On the basis of the reported annual production volumes in Europe (EFFA, 2004b, 2005b, 2006a, 2007b; Flavour Industry, 2009a,b) the estimated combined daily *per capita* intake as flavourings of the 17 candidate substances assigned to structural class I is approximately 1250 µg, which does not exceed the threshold of concern for a compound belonging to structural class I of 1800 µg per person per day.

For the 11 candidate substances assigned to structural class II the combined intake is approximately 15 µg, which does not exceed the threshold of concern for a compound belonging to structural class II of 540 µg/person per day.

The 29 candidate substances are structurally related to 26 supporting substances of which 23 were evaluated by JECFA at its 51st meeting (JECFA, 2000a) and three were evaluated by the JECFA at its 63rd meeting (JECFA, 2005b). The total combined intake (in Europe) of the candidate and the supporting substances all assigned to structural class I is approximately 25500 µg/*capita* per day, which exceeds the threshold of concern for the corresponding structural class of 1800/person per day.

For one of the supporting substances from structural class I, menthol [FL-no: 02.015], The JECFA established an acceptable daily intake (ADI) of 4 mg/kg bw per day at their 51st meeting (JECFA, 2000a). The combined intake of the candidate and supporting substances from structural class I of 25500 µg/*capita* per day ~ 0.425 mg/kg bw per day is approximately 10 times below this ADI.

In Europe, the total estimated combined intake of the candidate and the supporting substances assigned to structural class II is 16 µg/*capita* per day, which does not exceed the threshold of concern for the corresponding structural class (540 µg/person per day).

The intake of the one candidate substance assigned to structural class III is 27 µg/*capita* per 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 LD₅₀ values in rats, mice or rabbits ranged from 230 to 50000 mg/kg body weight (bw). The magnitudes of the LD₅₀ values indicate that the oral acute toxicity is low for the candidate and supporting substances.

The acute toxicity data are summarised in Appendix E, Table 16.

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], bisabol-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 Appendix E, Table 17).

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 per 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 B6C3F₁ 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 per day, respectively), increased incidence of nephropathy in females receiving 0.5 %, 1 % and 4 % (equivalent to 500, 1000 and 4000 mg/kg bw per day) and increased severity of nephropathy in males at all treatment doses (intakes of 250 mg/kg bw per 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 per day, respectively) and 4 % group females (equivalent to 10,000 mg/kg bw per day) (Lindamood et al., 1992; NTP, 1995). 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 and 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 per day) and for females 0, 0.25, 0.5 or 1 % (equivalent to 0, 180, 330 or 650 mg/kg bw per 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, 1995). 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, 1995).

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-2u-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, 1995); 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 B6C3F₁ 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 per day in males and 0, 510, 1020 or 2100 mg/kg bw per 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

per day (NTP, 1995). 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, 1995). 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 per 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 per day. No such changes were observed in females at the lower doses tested and therefore, 50 mg/kg bw per 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 per day should be regarded as the NOAEL for males (Dunster et al., 2007).

A NOAEL of 150 mg/kg bw per 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, 1994).

A NOAEL of 850 mg/kg bw per 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 per day of sclareol and two groups of 10 rats received dose levels of approximately 8.4 mg/kg bw per day of cedrol. The animals were dosed seven days per week via gavage. No adverse effects were observed (IOFI, 2006).

Data are available for six supporting substances [FL-nos: 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 E, Table 17).

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 per 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 24, 27 and 48 mg/kg bw per 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 per 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 per day) for 20 weeks (Hagan et al., 1967).

Since the publication of FGE.18Rev2 (EFSA CEF Panel, 2011) new data on myrcene have become available (Bauter, 2013). The results are described in more detail in FGE.78Rev2 (EFSA, 2015). In this FGE a NOAEL of 44 mg/kg bw per day was derived which can be used for the evaluation of [FL-no: 02.146] in the current FGE.

The repeated dose toxicity data are summarised in Appendix E, Table 17.

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 per 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 and Evans, 1982). In a second study, administration of 1557 mg/kg bw per 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 per 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 per 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 per day (Politano et al., 2008).

Data are available for three supporting substances [FL-nos: 02.013, 02.015 and 01.008] (See Annex E, Table 18).

A NOAEL of 365 mg/kg bw per day was reported for linalool [FL-no: 02.013]. A higher dose of 729 mg/kg bw per day resulted in decreased live litter size and increased pup mortality; maternal toxicity was observed at all doses tested (Hoberman and Christian, 1989). NOAELs of 185 to 425 mg/kg bw per 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 per day, 250 mg/kg bw per day and 300 mg/kg bw per day, respectively (Delgado et al., 1993a; Delgado et al., 1993b; Paumgartten et al., 1998).

The developmental/reproductive toxicity data are summarised in Appendix E, Table 18.

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for nine candidate [FL-nos: 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-nos: 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-nos: 02.052 and 02.123] and for three supporting substances [FL-nos: 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., 1988). 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, 1999). 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, 1999).

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, 1997). 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, 1995). 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, 1996). 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 LD₅₀ (Barilyak and 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 µg/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, 1983; 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 TA100 without metabolic activation (Rockwell and 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 Appendix E, Tables 19 and 20.

CONCLUSIONS

The present revision 3 of the Flavouring Group Evaluation 18 (FGE.18Rev3) is due to the availability of new toxicity data for myrcene, [FL-no: 01.008] which was used for the safety evaluation of the candidate substance (E)-3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146].

In this revision seven of the candidate substances are aliphatic saturated tertiary alcohols and one is an ester of such, five are aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two are esters of such, one is an aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond, one is an aliphatic unsaturated tertiary alcohol without terminal double bond, two are monocyclic saturated tertiary alcohols and one is an ester of such, two are monocyclic unsaturated tertiary alcohols, two are mono- and bicyclic unsaturated tertiary alcohols with an isolated terminal double bond, two are bicyclic unsaturated esters, two are bi- and tricyclic tertiary alcohols and one is a tertiary alcohol with an aromatic substituent.

Eighteen of the 29 candidate substances possess one or more chiral centres and/or can exist as geometrical stereoisomers due to the presence of a double bond: [FL-nos: 02.120, 02.129, 02.140, 02.144, 02.146, 02.147, 02.149, 02.150, 02.168, 02.197, 02.206, 02.226, 02.230, 02.253, 09.171, 09.614, 09.671 and 09.808]. For four of these substances [FL-nos: 02.146, 02.147, 02.168 and 02.197] the stereoisomeric composition has not been specified sufficiently.

Seventeen of the 29 candidate substances are classified into structural class I, eleven candidate substances are classified into structural class II and one is classified into structural class III according to the decision tree approach.

Twenty-one out of the 29 candidate substances have been reported to occur in a wide range of food items.

According to the default MSDI approach, 28 of the 29 flavouring substances in this group have intakes in Europe that vary from 0.0012 to 27 $\mu\text{g/capita}$ per day, which are below the thresholds of concern for structural classes I, II and III substance of 1800, 540 and 90 $\mu\text{g/person}$ per day, respectively. The remaining substance, terpineol [FL-no: 02.230] belonging to structural class I, has an MSDI of 1200 $\mu\text{g/capita}$ per 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 seventeen candidate substances belonging to class I and of eleven candidate substances belonging to class II would result in combined intakes of approximately 1250 and 15 $\mu\text{g/capita}$ per day, respectively. These values are lower than the thresholds of concern for structural class I and class II substances of 1800 and 540 $\mu\text{g/person}$ per day, respectively.

For flavouring substances belonging to structural class I, the total combined intake of the candidate and supporting substances is approximately 25500 $\mu\text{g/capita}$ per day. This total combined intake exceeds the threshold for structural class I of 1800 $\mu\text{g/person}$ per day. For one of the supporting substances from structural class I, menthol [FL-no: 02.015], the JECFA established an acceptable daily intake (ADI) of 4 mg/kg bw per day at their 51st meeting. The combined intake of the candidate and supporting substances from structural class I of 25500 $\mu\text{g/capita}$ per day \sim 0.425 mg/kg bw per day is approximately 10 times below this ADI. For flavouring substances belonging to structural class II, the total combined intake of the candidate and supporting substances is approximately 16 $\mu\text{g/capita}$ per day, which is below the threshold of concern for structural class II (540 $\mu\text{g/person}$ per 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 do not give rise to safety concerns with respect to genotoxicity.

Twenty-eight of the candidate substances are anticipated to be metabolised to innocuous products. For the candidate substance (E)-3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no metabolism data are available and therefore it cannot be predicted to be metabolised to innocuous products.

In Revision 2 of FGE.18, for substance (E)-3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] no appropriate NOAEL was available and additional data were requested. In the present revision of FGE.18 (Revision 3), new toxicity data on the supporting substance myrcene [FL-no: 01.008] became available, providing an appropriate NOAEL of 44 mg/kg bw per day which was used for the evaluation of the candidate substance [FL-no: 02.146]. Based on the MSDI intake of 12 µg/capita per day, the margin of safety calculated for [FL-no: 02.146] is 2.2×10^5 .

Therefore, none of the 29 candidate substances [FL-nos: 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 give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances, on the basis of the default MSDI approach.

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 25 candidate substances. However, information on purity criteria and/or stereoisomeric composition has not been specified sufficiently for four substances [FL-no: 02.129, 02.147, 02.168 and 02.197]. Thus, the final evaluation of the materials of commerce cannot be performed for these substances, pending further information.

Thus, for 25 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach: [FL-nos: 02.041, 02.052, 02.054, 02.120, 02.123, 02.140, 02.144, 02.146, 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].

The estimated intakes for fifteen of the seventeen candidate substances in structural class I, based on the mTAMDI approach, are 3900 µg/person per day, and for the two remaining substances 7000 and 14000 µg/person per day respectively. These seventeen mTAMDI are all above the threshold of concern of 1800 µg/person per day for a structural class I substance. For nine of the eleven substances assigned to structural class II, the mTAMDI are 3900 µg/person per day and for the remaining two substances, 110 and 1600 µg/person per day, respectively. For ten substances, the estimated intakes are above the threshold of concern for structural class II substances of 540 µg/person per day. For the one substance assigned to structural class III the mTAMDI is 5700 µg/person per day, which is above the threshold of concern for structural class III substances of 90 µg/person per day.

In conclusion, for all candidate substances except [FL-no: 02.146] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For four substances [FL-nos: 02.129, 02.147, 02.168, 02.197] additional information on purity criteria and/or stereoisomeric composition is required.

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Appendix A. Summary of Safety Evaluation

Table 7: Summary of Safety Evaluation Applying the Procedure

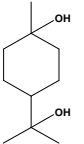
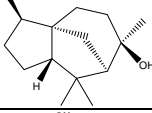
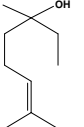
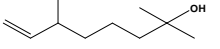
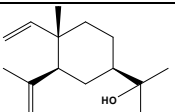
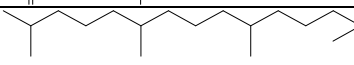
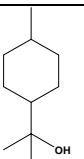
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.054	p-Menthane-1,8-diol		11	Class I A3: Intake below threshold	d	f	
02.120 2030	(+)-Cedrol		13	Class I A3: Intake below threshold	d	f	
02.140	1,2-Dihydrolinalool		0.044	Class I A3: Intake below threshold	d	f	
02.144	2,6-Dimethyloct-7-en-2-ol		0.0012	Class I A3: Intake below threshold	d	f	
02.149	(-)-alpha-Elemol		1.6	Class I A3: Intake below threshold	d	f	
02.168	Isophytol		0.037	Class I A3: Intake below threshold	d	g	
02.171	p-Menthan-8-ol		0.012	Class I A3: Intake below threshold	d	f	

Table 7: Summary of Safety Evaluation Applying the Procedure

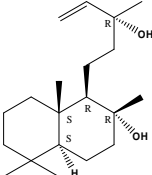
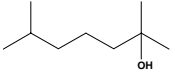
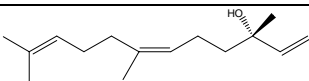
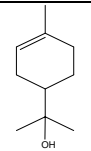
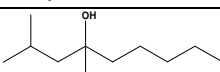
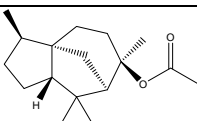
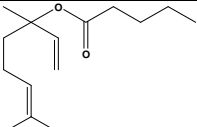
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.206 2029	(-)-Sclareol		0.67	Class I A3: Intake below threshold	d	f	
02.219	2,6-Dimethyl-2-heptanol		0.012	Class I A3: Intake below threshold	d	f	
02.226	[S-(cis)]-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol		0.049	Class I A3: Intake below threshold	d	f	
02.230	Terpineol	 alpha-Terpineol shown	1200	Class I A3: Intake below threshold	d	f	
02.253 1850	2,4-Dimethyl-4-Nonanol		0.24	Class I A3: Intake below threshold	d	f	
09.171	Cedryl acetate		0.99	Class I A3: Intake below threshold	d	f	
09.614	Linalyl valerate		0.43	Class I A3: Intake below threshold	d	f	

Table 7: Summary of Safety Evaluation Applying the Procedure

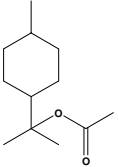
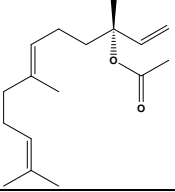
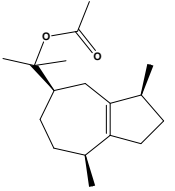



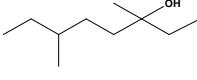
FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
09.617	p-Menthan-8-yl acetate		0.012	Class I A3: Intake below threshold	d	f	
09.671	(3S,6Z)-Nerolidyl acetate		0.061	Class I A3: Intake below threshold	d	f	
09.808	Guaiyl acetate		0.0012	Class I A3: Intake below threshold	d	f	
02.041	2-Methylbutan-2-ol		2.7	Class II A3: Intake below threshold	d	f	
02.052	2-Methylpropan-2-ol		0.012	Class II A3: Intake below threshold	d	f	
02.123	2-Methylbut-3-en-2- ol		0.0012	Class II A3: Intake below threshold	d	f	
02.147	3,6-Dimethyloctan-3- ol		0.0012	Class II A3: Intake below threshold	d	g	

Table 7: Summary of Safety Evaluation Applying the Procedure

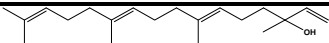
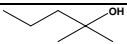
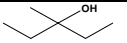
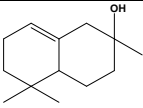
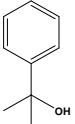
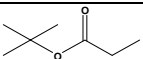
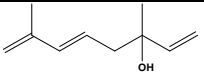
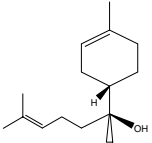
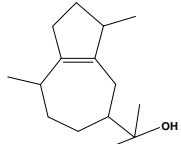
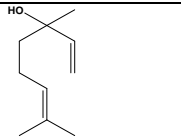
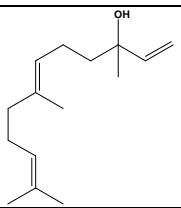

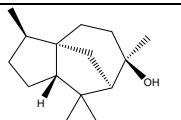
FL-no	EU Register name	Structural formula	MSDI ^a ($\mu\text{g/capita}$ per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.150	(E,E)-Geranyl linalool		0.026	Class II A3: Intake below threshold	d	f	
02.181	2-Methylpentan-2-ol		0.12	Class II A3: Intake below threshold	d	f	
02.184	3-Methylpentan-3-ol		0.0012	Class II A3: Intake below threshold	d	f	
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	d	g	
02.203	2-Phenylpropan-2-ol		0.0012	Class II A3: Intake below threshold	d	f	
09.356	1,1-Dimethylethyl propionate		0.0012	Class II A3: Intake below threshold	d	f	
02.146	(E)-3,7- Dimethylocta-1,5,7- trien-3-ol		12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		No safety concern at the estimated level of intake based on the MSDI approach.

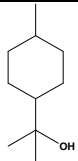

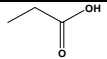
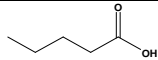
Table 7: Summary of Safety Evaluation Applying the Procedure

FL-no	EU Register name	Structural formula	MSDI ^a (µg/capita per day)	Class ^b Evaluation procedure path ^c	Outcome on the named compound ^{d,e}	Outcome on the material of commerce ^{f,g,h}	Evaluation remarks
02.129 2031	(l)-alpha-Bisabolol	 (l)-alpha-Bisabolol shown	27	Class III A3: Intake below threshold	d	g	
(a):	EU MSDI: Amount added to food as flavour in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.						
(b):	Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.						
(c):	Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.						
(d):	No safety concern based on intake calculated by the MSDI approach of the named compound.						
(e):	Data must be available on the substance or closely related substances to perform a safety evaluation.						
(f):	No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).						
(g):	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.						
(h):	No conclusion can be drawn due to lack of information on the purity of the material of commerce.						

EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 8: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status ^a JECFA status ^b CoE status ^c EFSA status	Structural class ^d Procedure path (JECFA) ^e	Comments
	Guaiol				Not evaluated as flavour
02.013	Linalool 356		No safety concern (JECFA, 2000a) Category A (CoE, 1992)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
02.018	Nerolidol 1646		Category B (CoE, 1992)	Class I A3: Intake below threshold	
02.052	2-Methylpropan-2-ol		Category B (CoE, 1992) FGE.18	Class II A3: Intake below threshold	FGE.18: No safety concern based on the intake calculation by the MSDI approach
02.120	(+)-Cedrol 2030		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
08.002	Acetic acid 81		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.003	Propionic acid 84		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 (SCF, 1995) No safety concern (JECFA, 1999) Category A (CoE, 1992)	Class I A3: Intake below threshold	

- (a): 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.
- (b): No safety concern at estimated levels of intake.
- (c): Category A: Flavouring substance which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.
- (d): Threshold of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.
- (e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

SUPPORTING SUBSTANCES SUMMARY

Table 9: Supporting Substances Summary

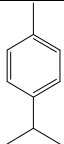
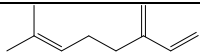
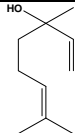
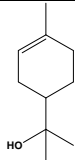
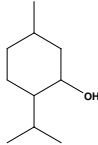
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
01.002	1-Isopropyl-4-methylbenzene		2356 620 99-87-6	1325 JECFA specification (JECFA, 2005a).	926	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	
01.008	Myrcene		2762 2197 123-35-3	1327 JECFA specification (JECFA, 2005a)	290	No safety concern (JECFA, 2005b) Category B (CoE, 1992)	EFSA conclusion: B4: Adequate NOAEL exists (EFSA, 2015).
02.013	Linalool		2635 61 78-70-6	356 JECFA specification (JECFA, 1998)	2200	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)
02.014	alpha-Terpineol		3045 62 98-55-5	366 JECFA specification (JECFA, 1998)	2600	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998)	16000	No safety concern (JECFA, 2000a) Category A	ADI: 0-4 (JECFA, 2000a).

Table 9: Supporting Substances Summary

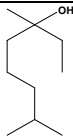
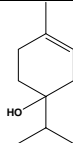
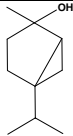
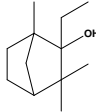
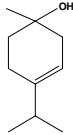
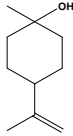
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
						(CoE, 1992)	
02.028	3,7-Dimethyloctan-3-ol		3060 77 78-69-3	357 JECFA specification (JECFA, 1998)	47	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.072	4-Terpinenol		2248 2229 562-74-3	439 JECFA specification (JECFA, 2000b)	150	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.085	Sabinene hydrate		3239 10309 546-79-2	441 JECFA specification (JECFA, 2000b)	0.91	No safety concern (JECFA, 2000a)	
02.095	2-Ethylfenchol		3491 10208 18368-91-7	440 JECFA specification (JECFA, 2001)	0.74	No safety concern (JECFA, 2000a)	
02.096	1-Terpinenol		3563 10252 586-82-3	373 JECFA specification (JECFA, 2000b)	35	No safety concern (JECFA, 2000a)	
02.097	beta-Terpineol		3564 10254 138-87-4	374 JECFA specification (JECFA, 2001)	1.3	No safety concern (JECFA, 2000a)	

Table 9: Supporting Substances Summary

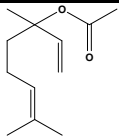
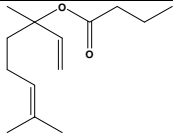
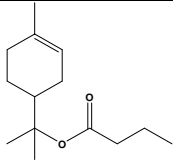
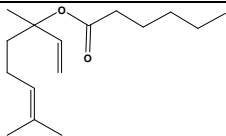
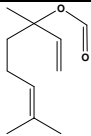
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.013	Linalyl acetate		2636 203 115-95-7	359 JECFA specification (JECFA, 1998)	1700	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)
09.050	Linalyl butyrate		2639 276 78-36-4	361 JECFA specification (JECFA, 1998)	8.4	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.052	Terpinyl butyrate		3049 278 2153-28-8	370 JECFA specification (JECFA, 2001)	5.1	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.068	Linalyl hexanoate		2643 318 7779-23-9	364 JECFA specification (JECFA, 1998)	0.85	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.080	Linalyl formate		2642 347 115-99-1	358 JECFA specification (JECFA, 2003)	6.9	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	GrADI: 0-0.5 (JECFA, 1980)

Table 9: Supporting Substances Summary

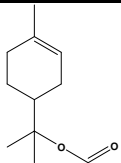
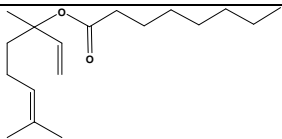
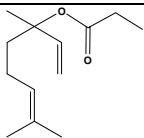
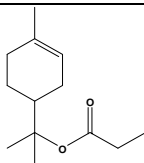
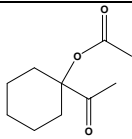
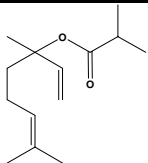
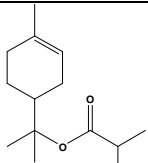
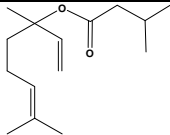
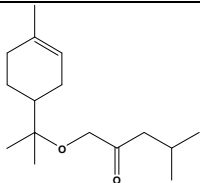
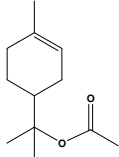
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09.081	alpha-Terpinyl formate		3052 348 2153-26-6	367 JECFA specification (JECFA, 2001)	0.12	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.116	Linalyl octanoate		2644 397 10024-64-3	365 JECFA specification (JECFA, 2000b)	0.12	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.130	Linalyl propionate		2645 411 144-39-8	360 JECFA specification (JECFA, 2003)	13	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.142	Terpinyl propionate		3053 423 80-27-3	369 JECFA specification (JECFA, 1998)	0.024	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.293	1-Acetoxy-1-acetylcyclohexane		3701 52789-73-8	442 JECFA specification (JECFA, 2001)	ND	Additional data required (JECFA, 2000a)	

Table 9: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) ^a (µg/capita per day)	SCF status ^b JECFA status ^c CoE status ^d	Comments
09.423	Linalyl isobutyrate		2640 298 78-35-3	362 JECFA specification (JECFA, 1998)	30	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.425	Terpinyl 2-methylpropionate		3050 300 7774-65-4	371 JECFA specification (JECFA, 2000b)	0.61	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.454	Linalyl isovalerate		2646 449 1118-27-0	363 JECFA specification (JECFA, 2000b)	4.6	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.461	Terpinyl isovalerate		3054 456 1142-85-4	372 JECFA specification (JECFA, 2001)	0.12	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.830	Terpineol acetate		3047 205 8007-35-0	368 JECFA specification (JECFA, 1998)	220	No safety concern (JECFA, 2000a)	

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

(b): 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.

- (c): No safety concern at estimated levels of intake.
- (d): Category A: Flavouring substance which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

Appendix B. Procedure for the Safety Evaluation

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

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 µg/person per day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

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 products¹⁶ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous¹⁷ (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.

¹⁶ "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).

¹⁷ "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).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

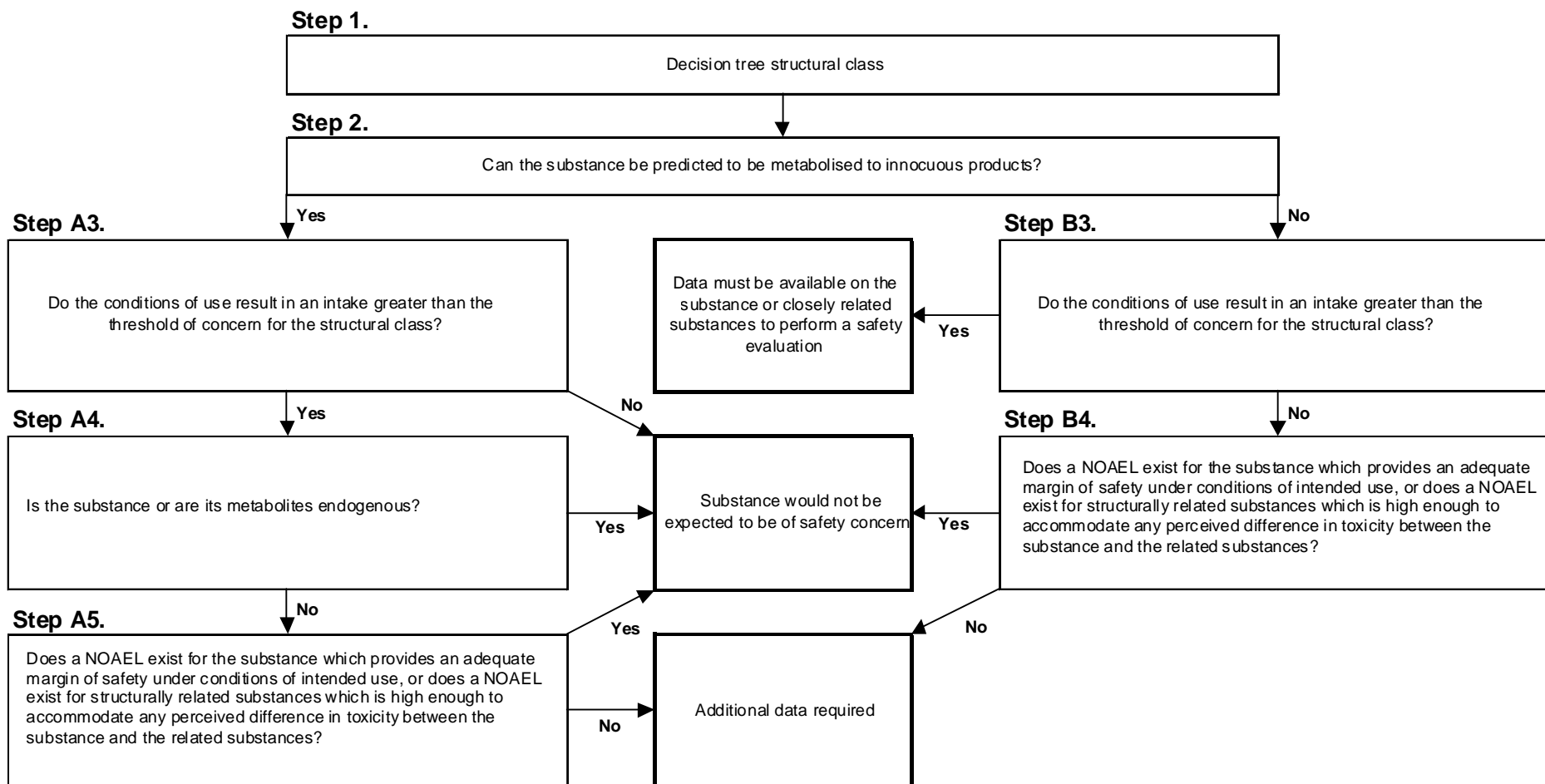


Figure A.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

Appendix C. Use Levels / mTAMDI

C.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table 10) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004c).

Table 10: Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000)

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

The “normal and maximum use levels” are provided by Industry for the 29 candidate substances in the present flavouring group (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012) (Table 11).

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFFA, 2005a; EFFA, 2006b; EFFA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.041	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
02.146	0,2	-	0,2	-	-	0,5	-	-	-	-	-	-	-	-	0,2	0,2	-	-
	2	-	2	-	-	5	-	-	-	-	-	-	-	-	2	2	-	-
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
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

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFFA, 2005a; EFFA, 2006b; EFFA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.171	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

Table 11: Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.18Rev3 (EFFA, 2005a; EFFA, 2006b; EFFA, 2007a)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
09.356	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

C.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 12. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table 12: 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 per day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table 13):

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

Table 13: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	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 14) are presented for the 29 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004a; EFFA, 2005b; EFFA, 2006a; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2009a; Flavour Industry, 2012). The mTAMDI values are only given for highest reported normal use levels (See Table 14).

Table 14: Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per 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	(-)-alpha-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	(3S,6Z)-Nerolidyl acetate	3900	Class I	1800
09.808	Guaiyl 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
02.147	3,6-Dimethyloctan-3-ol	3900	Class II	540
02.150	(E,E)-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
02.146	(E)-3,7-Dimethylocta-1,5,7-trien-3-ol	110	Class II	540
02.129	(l)-alpha-Bisabolol	5700	Class III	90

Appendix D. Metabolism

D.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 15).

Table 15: 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	
2	09.356	1,1-Dimethylethyl propionate	Aliphatic unsaturated tertiary alcohols with isolated terminal double bonds and two esters thereof
	02.123	2-Methylbut-3-en-2-ol	
	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	
3	09.671	Nerolidyl acetate	Aliphatic unsaturated tertiary alcohol with a conjugated terminal double bond
	02.146	3,7- Dimethylocta-1,5,7-trien-3-ol	
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-trimethylnaphthalen-2-ol	
	02.120	Cedrol	
	09.171	Cedryl acetate	
8	02.203	2-Phenylpropan-2-ol	Tertiary alcohol with an aromatic substituent

D.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 ($T_{1/2\alpha}$) about 3 min and the elimination half-life ($T_{1/2\beta}$) 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 $T_{1/2\beta}$ 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 intravenoustreated 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 hour period. Very low levels (0.04 %) of oxidised metabolites were found in bile after 3 hours, including 3- α -hydroxysclareol (0.24 %), 3- β -hydroxysclareol (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, 2 α -hydroxysclareol, 3 β -hydroxysclareol, 18-hydroxysclareol, 2 α ,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 and Srivatsan, 1988). In a minor pathway, the double bond 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 and 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).

[1,2-¹⁴C]-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 CO₂ 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., 1974).

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, 1928) 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 1-menthol was administered orally to two human volunteers followed by oral or intravenous administration of 200 mg [6-¹³C]-glucuronolactone or [6-¹³C]-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 1-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 and 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 hours, 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.

D.3 Biotransformation

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 ($t_{1/2} = 121$ min) hydrolysed to a mixture of linalool and the ring-closed isomer alpha-terpineol. In acidic artificial gastric juice, linalyl acetate is rapidly hydrolysed ($t_{1/2} < 5$ min) to yield linalool (Buck and Renwick, 1998). Linalyl acetate was slowly hydrolysed ($t_{1/2} = 153 - 198$ 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. $> 5 \text{ min}^{-1}$ 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 and 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, 1974).

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])

[2-¹³C]-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 ¹³C-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-hour 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., 1953).

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., 1953).

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 ¹³C-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 D.1.)

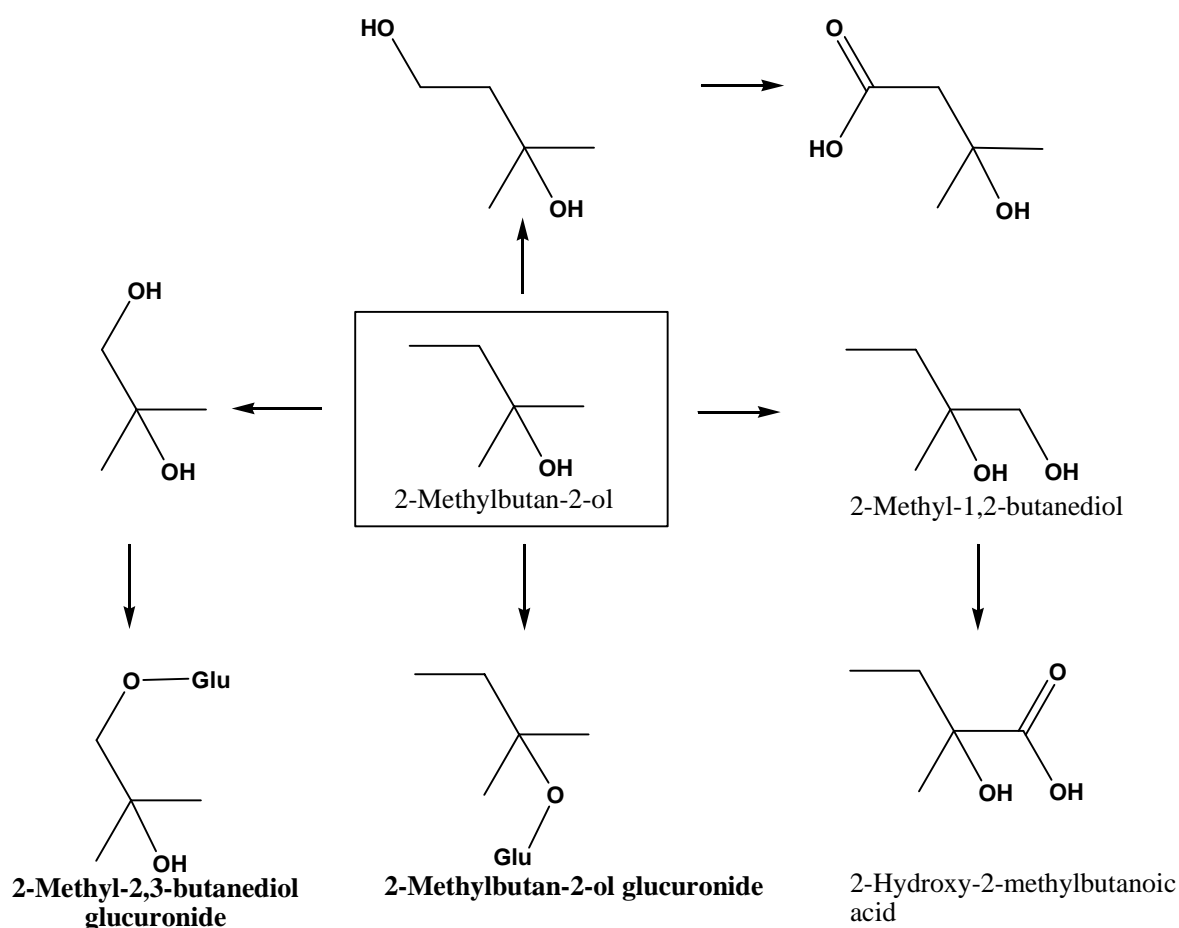


Figure D.1. Metabolism of 2-methylbutan-2-ol (tert-amyl-alcohol) in rats (Amberg et al., 1999). Main metabolism products are in bold.

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., 1953).

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.

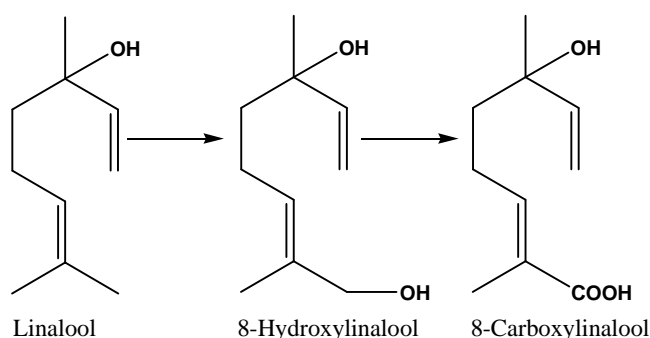


Figure D.2 Metabolism of linalool (Chadha & Madyastha 1984).

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, 1974).

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 and Madyastha, 1984) (Figure D.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.

Subgroup 3: Aliphatic unsaturated tertiary alcohols with a conjugated terminal double bond

No metabolism studies are found for the candidate substance, 3,7-dimethylocta-1,5,7-trien-3-ol, [FL-no: 02.146]. For the supporting substance, myrcene, two studies on metabolism are found.

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 and 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 D.3).

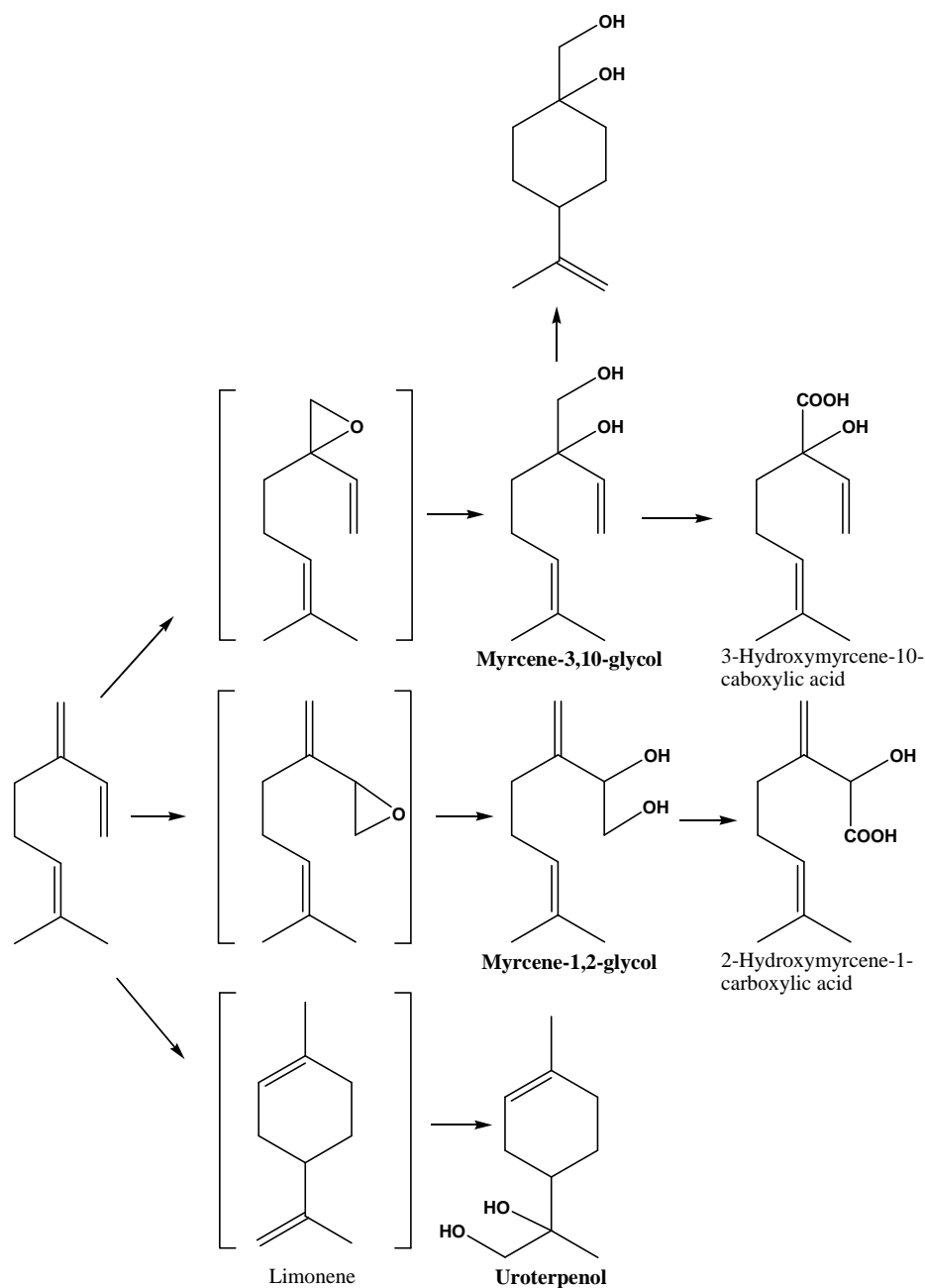


Figure D.3 Metabolism of myrcene (Ishida et al., 1981 and Madyastha & Srivatsan 1987). Intermediate products are in brackets and main metabolism products are in bold.

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, 1974). *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 glucuronides (Rahman, 1974).

Subgroup 5: Monocyclic saturated tertiary alcohols and esters

There are no metabolism studies on the candidate alicyclic substances p-menthane-1,8-diol, bisabolol, 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 D.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 and Srivatsan, 1988).

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 and Srivatsan, 1988; 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-carboxylic 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 and Srivatsan, 1988) supports the finding of p-menthane-3,8-diol and 3,8-dihydroxy-p-menthane-7-carboxylic 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.

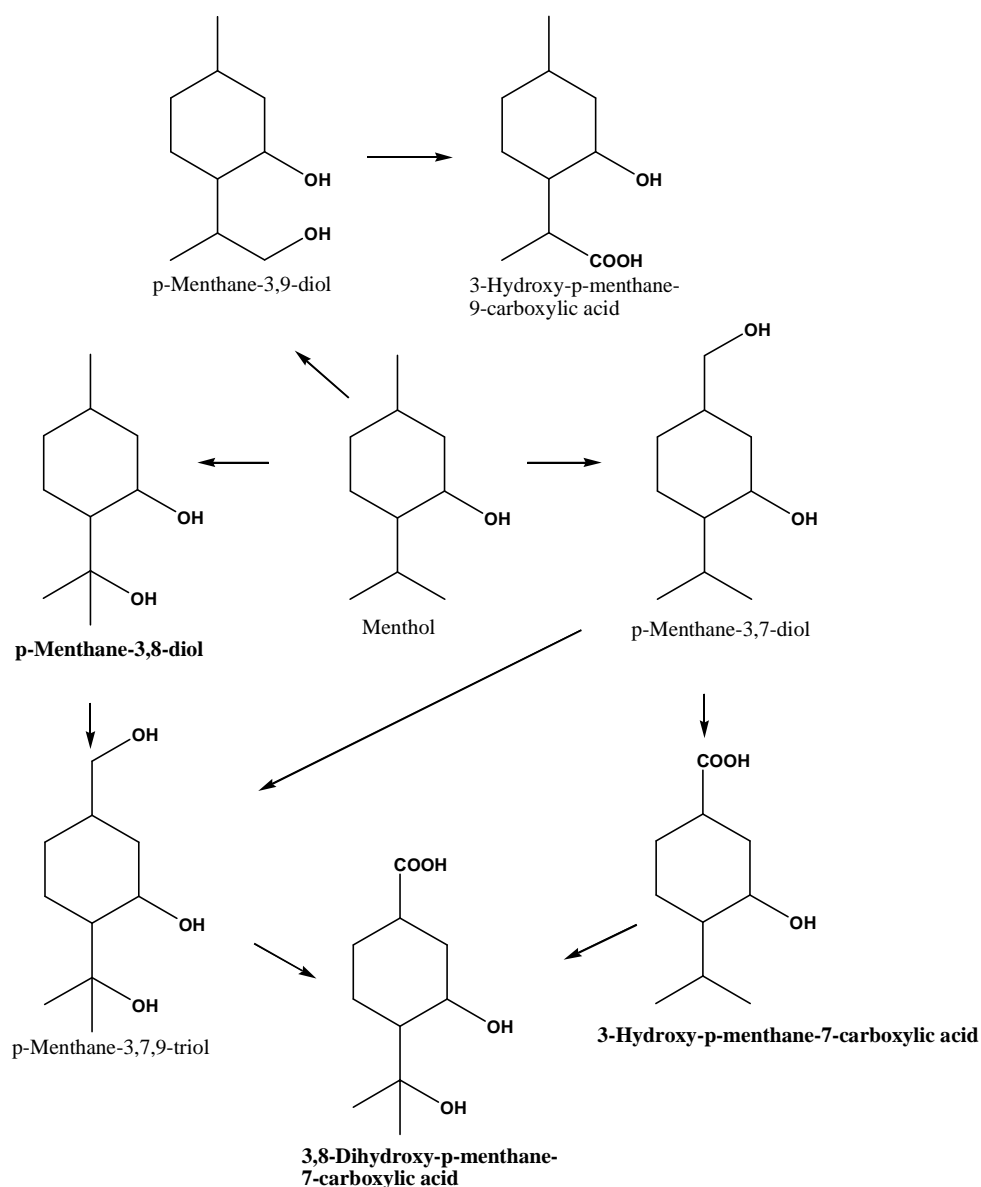


Figure D.4. Metabolism of menthol in rats (Yamaguchi et al., 1994). Main metabolism products are in bold.

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.

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-trimethylnaphthalen-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 % methyl-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 and 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 cedrol (-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):

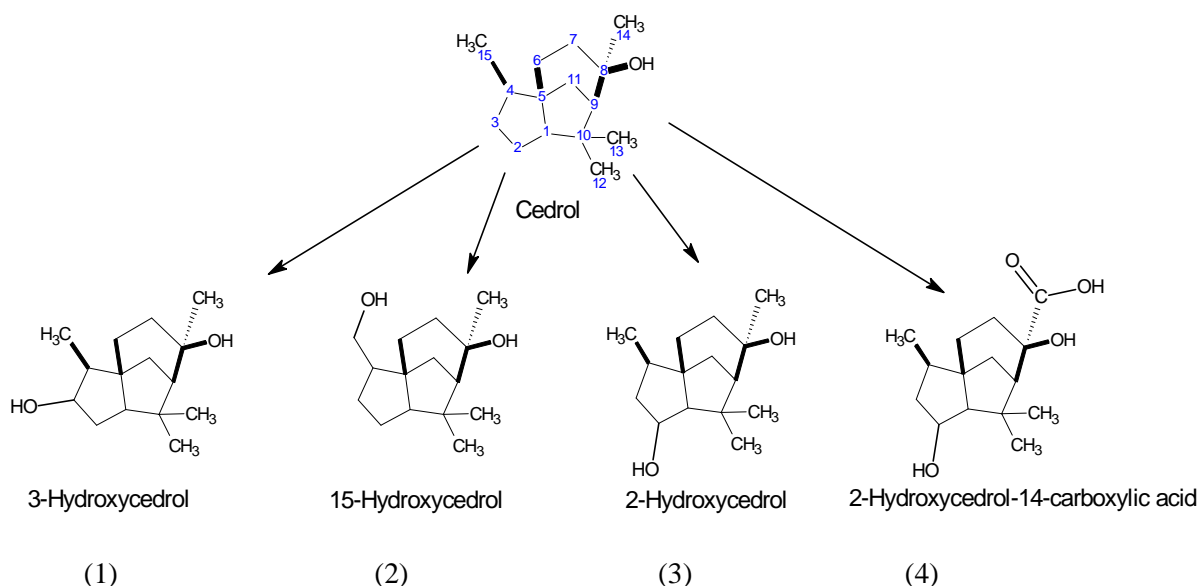


Figure D.5 Metabolism of cedrol in a dog treated with phenobarbital. The carboxylic acid is presumably formed from 2-hydroxycedrol, but this was not further studied (after Trifilieff et al, 1975).

Subgroup 8: 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-hydroxypropionic 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 and 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-tolylpropan-2-ol (39 % of recovered dose) and 2-p-carboxyphenylpropan-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).

Oxidation of terminal double bonds

Ten candidate substances [FL-no: 02.123, 02.168, 02.144, 02.146, 02.150, 02.206, 02.226, 09.614, 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 bonds, indicating that terminal double bonds as such will not give rise to intermediate epoxides.

D.4 Summary and Conclusion

Six candidate flavouring substances in this group are esters [FL-no: 09.171, 09.356, 09.614, 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 carboxylic 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 substance 3,7-dimethylocta-1,5,7-trien-3-ol, [FL-no: 02.146]. This substance also has an alcohol moiety 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 glucuronides. 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.

For the remaining candidate substance [FL-no: 02.146] (subgroup 3), which contains a conjugated terminal double bond, data from the supporting substance myrcene [FL-no: 01.008] indicate that this terminal double bond may be oxidised, giving rise to epoxide intermediates. Thus, it cannot be anticipated that this candidate substance 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.

Appendix E. Toxicity

Table 16: 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 and Brown, 1952)	
	Rat	M, F	Gavage	3046	(Johnson, 1981a)	
	Rat	M, F	Gavage	2733	(Johnson, 1981b)	
	Rat	NR	Oral	> 3800	(Eastman Kodak Co., 1994)	
	Rabbit	M, F	Gavage	3560	(Munch, 1972)	
2-Methylbutan-2-ol [02.041]	Rat	NR	Oral	1000	(Schaffarzick and Brown, 1952)	
	Rat	NR	Oral	1000 – 2000	(Dow Chemical Company, 1982)	
	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, 1979)	
	Rat	NR	Oral	> 5000	(Moreno, 1976a)	
3,6-Dimethyloctan-3-ol [02.147]	Rat	NR	Oral	> 5000	(Moreno, 1973a)	
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., 1992)	
	Mouse	M, F	Oral	3918	(Hoffman-LaRoche, Inc., 1967)	
	Mouse	M, F	Oral	3000 ¹ 1700 ²	(Rhône-Poulenc, Inc., 1992)	
(3,7-Dimethyloctan-3-ol [02.028])	Rat	NR	Oral	> 5000	(Moreno, 1976b)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat	NR	Oral	3600	(Moreno, 1973b)	
Myrcenol [02.185]	Rat	NR	Oral	5300	(Moreno, 1972a)	
Ocimenol [02.191]	Rat	NR	Oral	1700	(Wohl, 1974)	
Geranyl linalool [02.150]	Rat	NR	Oral	> 5000	(Moreno, 1982a)	
	Mouse	M, F	Oral	14,632	(Hoffman-LaRoche Inc., 1967)	
(Linalyl formate [09.080])	Rat	NR	Oral	> 5000	(Russell, 1973)	
	Mouse	M	Oral	5389	(Hoffman-LaRoche, Inc., 1967)	
(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., 1967)	
(Linalyl propionate [09.130])	Rat	NR	Oral	> 5000	(Moreno, 1973c)	
	Mouse	M	Oral	13,874	(Hoffman-LaRoche, Inc., 1967)	
(Linalyl butyrate [09.050])	Rat	NR	Oral	> 5000	(Levenstein, 1975)	
	Mouse	M	Oral	> 8907	(Hoffman-LaRoche, Inc., 1967)	
(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., 1967)	
(Linalyl isovalerate [09.454])	Rat	NR	Oral	> 5000	(Moreno, 1975)	
	Mouse	M, F	Oral	25,165	(Hoffman-LaRoche, Inc., 1967)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Linalyl hexanoate [09.068])	Mouse	M, F	Oral	37,869	(Hoffman-LaRoche Inc., 1967)	
Myrcenyl acetate [09.669]	Rat	NR	Oral	6300	(Moreno, 1972b)	
(Linalyl octanoate [09.116])	Mouse	M, F	Oral	48,849	(Hoffman-LaRoche Inc., 1967)	
Nerolidyl acetate [09.671]	Rat	NR	Oral	> 5000	(Moreno, 1976c)	
(alpha-Terpineol [02.014])	Rat	NR	Oral	4300 ³	(Moreno, 1971)	
	Mouse	M	Gavage	2830	(Yamahara et al., 1985)	
(alpha-Terpinyol formate [09.081])	Rat	NR	Oral	> 5000	(Moreno, 1976d)	
(Terpinyl acetate [09.830])	Rat	M, F	Gavage	5075	(Jenner et al., 1964)	
(Terpinyl propionate [09.142])	Rat	NR	Oral	> 5000	(Moreno, 1973d)	
(Terpinyl 2-methylpropionate [09.425])	Rat	NR	Oral	> 5000	(Moreno, 1982b)	
(4-Terpinenol [02.072])	Rat	NR	Oral	1300	(Moreno, 1977)	
Guaiyl acetate [09.808]	Rat	NR	Oral	> 5000	(Moreno, 1973e)	
2-Phenylpropan-2-ol [02.203]	Rat	NR	Oral	1037 ⁴	(Patty, 1982)	
(beta-Terpineol) [02.097])	Rat	NR	Oral	4300 ³	(Moreno, 1971)	
<i>p</i> -Menthan-8-ol [02.171]	Rat	NR	Oral	> 5000	(Moreno, 1973f)	
(1-Acetoxy-1-acetylcyclohexane [09.293])	Rat	M, F	Gavage	2150	(Piccirillo and Hartman, 1980)	

Table 16: Acute Toxicity

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Menthol [02.015])	Mouse	M	Gavage	2652	(Food and Drug Research Laboratories, Inc., 1975)	
	Mouse	M	Gavage	4384	(Food and Drug Research Laboratories, Inc., 1975)	
	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., 1975)	
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)	

¹Value represents calculated LD₅₀ when substance was administered in peanut oil.

²Value represents calculated LD₅₀ when substance was administered as an emulsion in an aqueous Arabic gum solution at 10 %.

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

⁴LD₅₀ value reported as 1.07 ml; conversion based on a density of 0.97 g/ml.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per 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 per 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 per day	90 days	None	(Lindamood et al., 1992); (NTP, 1995)	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 per 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 ^(d)	Drinking water	Males: 0, 0.125, 0.25 or 0.5 %, equivalent to 0, 90, 200 or 420 mg/kg bw per day Females: 0, 0.25, 0.5 or 1 %, equivalent to 0, 180, 330 or 650 mg/kg bw per day	2 years	None	(Cirvello et al., 1995); (NTP, 1995)	Fully described NTP study. No NOAELs. NTP conclusions on carcinogenicity: some evidence of carcinogenic activity in males, no evidence of carcinogenicity in females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
	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 per day	90 days	Male: 0.5 %, equivalent to 500 mg/kg bw per day Female: 1 %, equivalent to 1000 mg/kg bw per day	(Brown and 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 per day	90 days		(Brown and 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 per day	90 days	Male: 1 %, equivalent to 2500 mg/kg bw per day Female: 2 %, equivalent to 5000 mg/kg bw per day	(Lindamood et al., 1992); (NTP, 1995)	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 per day in males and 0, 510, 1020 or 2100 mg/kg bw per day in females	2 years	M: None F: 0.5 %, equivalent to 510 mg/kg bw per day	(Cirvello et al., 1995); (NTP, 1995)	Fully described NTP study. NTP conclusions on carcinogenicity: equivocal evidence of carcinogenic activity in males, some evidence of carcinogenic activity in females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
2-Methylbut-3-en-2-ol [02.123]	Rat; M, F 10	Gavage	0, 30, 150 or 750 mg/kg bw per day 5 days/week	4 weeks	150	(BASF, 1994)	Full study details provided. Study considered valid. Dosing only 5 days/week but a clear NOAEL.
(Linalool [02.013])	Rat; M, F NR	Diet ^(b)	0 or 50 mg/kg bw per day ^(a)	84 days	50 ^(a)	(Oser, 1967)	Unpublished report. Administered as 50 % mixture of linalool and citronellol. Slight retardation of growth in males at 50 mg/kg bw per day, not related to food intake.
	Mouse; F 30	Gavage	0 or 365 mg/kg bw per day	5 days	375 ^{(a),(c)}	(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 per day ^(a)	84 days	24 ^(a)	(Oser, 1967)	Unpublished report. Administered as mixture of linalyl acetate (24 mg/kg bw per day), linalyl isobutyrate (27 mg/kg bw per day) and geranyl acetate (48 mg/kg bw per day). Slight growth retardation of females.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
(Linalyl isobutyrate [09.423])	Rat; M, F NR	Diet ⁶	0 or 27 mg/kg bw per day ^(a)	84 days	27 ^(a)	(Oser, 1967)	
	Rat; NR 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw per 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 per day	17 weeks	500 ^(a) (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, 1987)	
	Rat; M, F 50	Gavage		103 weeks	1000 (710; 290) ⁸	(NTP, 1987)	
(Myrcene [01.008])	Rat; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day	13 weeks	None	(NTP, 2004)	Draft results only available. Evaluated by the JECFA. No NOAEL as most sensitive adverse effect (nephropathy) present at lowest dose tested (JECFA, 2006).
	Mouse; M, F 10	Gavage	0, 250, 500, 1000, 2000 or 4000 mg/kg bw per day, 5 days/week	13 weeks	Male: None Female: 250 mg/kg bw per day	(NTP, 2004)	Draft results only available. Evaluated by the JECFA. Liver hypertrophy in males at all doses (JECFA, 2006).

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
	Rats; M, F 10	Diet	0, 8.0, 40 and 44 mg/kg bw per day (males) 0, 9.6, 48 and 53 mg/kg bw per day (females)	90 days	Male: 44 mg/kg bw per day Female: 53 mg/kg bw per day	(Bauter, 2013)	Compliant with OECD Guideline 408.
(Menthol [02.015])	Mouse; M, F 50	Diet	0, 2000 or 4000 ppm, equivalent to 0, 300 or 600 mg/kg bw per day	103 weeks	600 ^(a)	(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., 1983)	Relative good quality.
	Rat; M, F 80	Diet	0, 100 or 200 mg/kg bw	5.5 weeks	200 ^(a)	(Herken, 1961)	Limited information.
	Rat; M, F 50	Diet	0, 3750 or 7500 ppm, equivalent to 0, 188 or 375 mg/kg bw per day	103 weeks	375 ^(a)	(National Cancer Institute, 1979)	Good quality.
(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 per day	20 weeks	500 ^(a)	(Hagan et al., 1967)	Very limited details provided.

Table 17: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw per day)	Reference	Comments
Sclareol [02.206]	Rat; M, F 20	Gavage	8.8 mg/kg bw per day	28 days	8.8	(IOFI, 2006)	
Cedrol [02.120]	Rat; M, F 20	Gavage	8.4 mg/kg bw per day	28 days	8.4	(IOFI, 2006)	
2,6-Dimethyloct-7-en-2-ol [02.144]	Rat; M, F 40	Gavage	10, 50, 500, 1000 mg/kg bw per 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

(a): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(b): Administered with citronellol as part of a 50/50 mixture.

(c): Immunotoxicity study.

(d): Three treatment groups per sex. Each dose group included 60 male and female animals.

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

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

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

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

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

Table 18: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Duration	Species/Species ex No/group	Route	Dose levels	NOAEL (mg/kg per day)	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, 3 400, 4 900 and 6 400 mg/kg bw per day)	Maternal: 0.5 % Foetal: None	(Daniel and Evans, 1982)	Study considered valid. Dose related reduction of offspring performance in neurobehavioural tests (righting reflex, negative geotaxis, open field behaviour, cliff avoidance, roto-rod performance).
	Developmental Toxicity: Gestation Days 6– 18	Mouse; F 9 - 12	Gavage ^(a)	0 or 10.5 mmol/kg bw per day, equivalent to 1 557 mg/kg bw per 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 ^(c)	Rat; F 10	Gavage	0, 250, 500 or 1 000 mg/kg bw per day coriander oil, equivalent to 0, 182.3, 364.5 or 729 mg/kg bw per day linalool	Maternal: None Foetal: 364.5	(Hoberman and 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 ^(b)	(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 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	

Table 18: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Duration	Species/Species ex No/group	Route	Dose levels	NOAEL (mg/kg per day)	Reference	Comments
	Teratology Gestation days 6 - 15	Hamster; F 20 - 22	Gavage	0, 4.05, 21.15, 98.2, 405	405 ^(b)	(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 ^(b)	(Food and Drug Research Laboratories, Inc., 1973)	
(Myrcene [01.008])	Teratology Gestation days 6 - 15	Rat; F 16 - 29	Gavage	0, 250, 500 and 1 200 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal: 500 mg/kg bw per day	(Delgado et al., 1993a)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Gestation day 15 to postnatal day 21	Rat; F 12 - 18	Gavage	0, 250, 500, 1 000 and 1 500 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal/neonatal: 250 mg/kg bw per day	(Delgado et al., 1993b)	Study considered valid. Evaluated by the JECFA.
	Reproductive and developmental toxicity: Prior to mating until postnatal day 21 ^(d)	Rat; M, F 60	Gavage	0, 100, 300, 500 mg/kg bw per day	Maternal/paternal: 500 mg/kg bw per day Foetal: 300 mg/kg bw per day	(Paumgartten et al., 1998)	Study considered valid. Evaluated by the JECFA.
2,6-Dimethyloct-7-en-2-ol [02.144]	Developmental Toxicity: Gestation Days 7 - 17	Rat, M, F 25	Gavage	0, 250, 500 and 1 000 mg/kg bw per day	Maternal: 500 mg/kg bw per day Foetal: 500 mg/kg bw per day	(Politano et al., 2008)	

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

(b): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(c): Animals were dosed for seven days prior to mating, during mating (maximum of seven days), during gestation, delivery and four days post parturition.

(d): 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

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	0.1, 0.5, 2.5, 5, 10 μl/plate (7800 μg/plate)	Questionable ^(a)	(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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	probably 1 to 10 μl/plate (7800 μg/plate) (corresponding pages of the report are lacking)	Questionable ^(a)	(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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	10000 μg/plate	Negative ^(a)	(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.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Yeast mitochondrial mutation assay	Several <i>Saccharomyces strains</i>	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 ^(a)	(McGregor et al., 1988)	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.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration	Chinese hamster ovary cells	160 - 5000 µg /ml	Negative	(NTP, 1984)	Limited validity. This non-GLP study was in accordance with OECD guideline 473 (1983) except that only a single harvest time 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 (15 600 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 (15 600 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

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
2-Methylbutan-2-ol [02.041]	Mutagenicity assays	<i>S. typhimurium</i> TA1535; TA1537; TA1538; <i>S. cerevisiae</i>	NR	Negative	(Dow Chemical Company, 1982)	Very short abstract only.
	Sister chromatid exchange	Chinese hamster ovary cells	160, 500, 1600, 5 000 µg/ml	Positive ^(c) Negative ^(b)	(NTP, 1997)	
	Sister chromatid exchange	Chinese hamster ovary cells	2 000, 3 000, 4 000, 5 000 µg/ml	Negative ^(a)	(NTP, 1997)	
2-Methylbut-3-en-2-ol [02.123]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	20 – 5 000 µg/plate	Negative ^(a)	(BASF, 1989)	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	<i>S. typhimurium</i> TA98; TA100	20 - 5 000 µg/plate	Negative ^(a)	(BASF, 1991)	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	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535	100, 333, 1 000, 3 333, 10 000 µg/plate	Equivocal ^(a)	(NTP, 1994)	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	<i>S. typhimurium</i> TA97, TA98, TA100, and TA1535	100 - 10000 µg/plate	Negative	(NTP, 2000)	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.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Linalool [02.013])	Ames test (modified)	<i>S. typhimurium</i> TA100	3 µl/2 ml (2 610 µg/2ml) incubation volume 1 mg/plate	Negative ^(a)	(Eder et al., 1980)	
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537	(1 000 µg/plate)	Negative ^(a)	(Ishidate et al., 1984)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	100 µl (87 000 µg)	Negative ^(a)	(Rockwell and Raw, 1979)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 nl/plate (8700 µg/plate)	Negative ^(a)	(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	250 µg/ml	Negative ^(a)	(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	<i>E. coli</i> WP2 <i>uvrA</i>	1 mg/plate (1 000 µg/plate)	Negative	(Yoo, 1986)	In Japanese (only summary and tables in English). Thus, the validity cannot be evaluated.
	Rec assay	<i>B. subtilis</i> H17(rec+); M45(rec-)	17 µg	Negative	(Oda et al., 1979)	
	Rec assay	<i>B. subtilis</i> 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.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell mutation	Mouse lymphoma L5178Y TK+/-	3.9 - 300 nl/ml	Negative ^(b) Positive ^(c)	(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	1 000 µM (15 4250 µg)	Negative ^{(a),(d)}	(Sasaki et al., 1989)	
(Linalyl acetate [09.013])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	25 000 nl/plate (22 575 µg/plate)	Negative ^(a)	(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	<i>B. subtilis</i> H17 (rec+); M45 (rec-)	18 µg	Negative	(Oda et al., 1979)	
	Chromosome aberration	Peripheral human lymphocytes	180 µg/ml	Negative ^(a)	(Bertens and van de Waart, 2000)	
(alpha-Terpineol [02.014])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 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	<i>S. typhimurium</i> TA97a; TA98; TA100;	2 500 µg/plate	Negative	(Gomes-Carneiro et al., 1998)	The study is considered valid. A slight but dose-related response was noted with TA102 with and without the use of metabolic activation.
		TA102	2 500 µg/plate	Weakly positive ^(e)		

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	1 000 µg/plate	Negative ^(a)	(National Cancer Institute, 1983)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10 000 µg/plate	Negative ^(a)	(Lorillard, 1983)	
	Spot test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmol/plate (463 µg/plate)	Negative ^(a)	(Florin et al., 1980)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	0.5 µl/ml (467µg/ml) 0.75µl/ml (700 µg/ml)	Negative ^(c) Negative ^(b)	(Kirby et al., 1984)	
	Mammalian cell mutation	Mouse lymphoma L5178Y TK +/-	300 nl/ml (280 µg/ml) 250 nl/ml (233 µg/ml)	Negative ^(a)	(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 ^(b) Negative ^(c)	(Lorillard, 1982)	
	Rec assay	<i>S. cerevisiae</i>	NR	Negative	(Oda et al., 1979)	
(Terpinyl acetate [09.830])	Rec assay	<i>B. subtilis</i> H17; M45	19 µg	Negative	(Oda et al., 1979)	
(beta-Terpineol) [02.097])	Ames	<i>S. typhimurium</i> TA98; TA100	0.05 µl (100 µl)	Negative ^(a)	(Rockwell and Raw, 1979)	
	Rec assay	<i>S. cerevisiae</i>	NR	Negative ^(a)	(Oda et al., 1979)	Article does not specify alpha- or beta-terpineol.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(1-Isopropyl-4-methylbenzene [01.002])	<i>In vivo/in vitro</i> Ames test	<i>S. typhimurium</i> TA98 and TA100	0.5 ml (equivalent to 1 706 mg/kg bw) administered to Sprague-Dawley rats, urine collected and tested <i>in vitro</i>	Negative ^(e)	(Rockwell and Raw, 1979)	
(Myrcene [01.008])	Ames test	<i>S. typhimurium</i> TA100; TA1535; TA97; TA98	0, 33, 100, 333, 1 000, 3 333 and 10 000 µg/plate	Negative ^(a)	(NTP, 1999)	
	Chromosome aberration	Human lymphocytes	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	Sister chromatid exchange	Human lymphocytes	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	HPRT assay	V79 Chinese hamster cells	0, 100, 500 and 1 000 µg/ml	Negative ^(a)	(Kauderer et al., 1991)	
	Sister chromatid exchange	V79 Chinese hamster cells	0, 100, 250 and 500 µg/ml	Negative ^(a)	(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	<i>S. typhimurium</i> , TA92, TA100, TA94, TA98, TA1535, TA1537	0 – 5 000 µg/plate	Negative ^(a)	(Ishidate et al., 1984)	d,l-Menthol was used. The study is considered valid.
	Ames test (preincubation method)	<i>S. typhimurium</i> , TA1535, TA97, TA100, TA98	3 - 666 µg/plate	Negative ^(a)	(Zeiger et al., 1988)	d,l-Menthol was used. The study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> , TA2637, TA100, TA98	0 and 5 - 500 µg/plate	Negative ^(a)	(Nohmi et al., 1985)	d,l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> , TA2637, TA100, TA98	0 and 20 - 500 µg/plate	Negative ^(a)	(Nohmi et al., 1985)	l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	<i>S. typhimurium</i> , TA1537, TA1535, TA100, TA98	0, 6.4, 32, 160 and 800 µg/plate	Negative ^(a)	(Andersen and Jensen, 1984)	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	<i>E. coli</i> WP2 <i>uvrA</i> (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	<i>S. typhimurium</i> TA97A; TA98; TA100; TA102	0, 5 - 800 µg/plate	Negative ^(a)	(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	<i>B. subtilis</i> H17, M45	Up to 10 000 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.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay	<i>B. subtilis</i> 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 and 0.1 - 1.3 mM (203.2 µg/ml ^(d))	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; 0 and 2 - 25 µg/ml ^(c) 0 and 16 - 167 µg/ml ²	Negative ^(a)	(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 and 10 mM (1 563 µg/ml ^(d))	Negative ^(a)	(Murthy et al., 1991)	The study is considered valid.
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1 and 10 µg/ml	Negative	(Food and Drug Research Laboratories, Inc., 1975)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 - 200 µg/ml	Negative ^(c)	(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 and 50 - 250 µg/ml	Negative ^(a)	(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.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosome aberration	Human lymphocytes	0, 0.1, 1 and 10 mM (1 563 µg/ml ^(d))	Negative ^(a)	(Murthy et al., 1991)	The study is considered valid.
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0 and 12.5 - 200 µg/ml	Negative ^(a)	(Myhr and 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	<i>S. typhimurium</i> , TA98, TA100, TA102, TA1535, TA1537	0 and 0.5 – 1 500 µg/plate	Negative ^(a)	(King and Harnasch, 2002)	
	Ames test	<i>S. typhimurium</i> , TA97a, TA98, TA100, TA1535	0 and 1 - 400 µg/plate in ethanol	Negative ^(a)	(Gomes-Carneiro et al., 2005)	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	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537	0 and 20 – 5 000 µg/plate in DMSO	Negative ^(a)	(CIR, 1999)	Review.
	Chromosome aberration	Chinese hamster fibroblast cells V79	0 and 0.78 - 40 µg/ml in DMSO	Negative ^(a)	(CIR, 1999)	Review.
Cedrol [02.120]	Ames test	<i>S. typhimurium</i> , TA97a, TA98, TA102, TA1535	0 – 5 000 µg/plate	Negative ^(a)	(Scheerbaum, 2001)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.
1,2-Dihydrolinalool [02.140]	Ames test	<i>S. typhimurium</i> , TA97, TA98, TA100, TA102, TA1535	0 – 1 000 µg/plate	Negative ^(a)	(Gocke, 1999)	Research report according to OECD and GLP-guidelines, the study is considered valid.
2,6-Dimethyloct-7-en-2-ol [02.144]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA102, TA1535, TA1537	0 – 5 000 µg/plate	Negative ^(a)	(King, 2000)	Research report according to OECD guideline 471 and GLP-guidelines, the study is considered valid.

Table 19: Genotoxicity (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
1,2,3,4,4a,5,6,7-Octahydro-2,5,5-trimethylnaphthalen-2-ol [02.197]	Ames test	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537 <i>E. coli</i> WP2 <i>uvrA</i>	0 - 250 µg/plate ^(c) 0 - 500 µg/plate ^(b)	Negative	(Watanabe, 2002)	Research report according to OECD guideline 471, the study is considered valid.
NR:	Not Reported.					
(a):	With and without metabolic activation.					
(b):	With metabolic activation.					
(c):	Without metabolic activation.					
(d):	With and without pre-treatment with mitomycin C at 0.15 microM for 21 hours.					
(e):	With and without presence of beta-glucuronidase.					

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
2-Methylpropan-2-ol [02.052]	Chromosomal Aberration assay	Male rats	Once via gavage	0.2xLD50	Positive ¹	(Barilyak and 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 ^(b) .
	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, 1996)	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.
	Micronucleus assay	Rat bone marrow cells	I.P. x 3 at 24 hours intervals (=72 h)	0, 39 – 1250 mg/kg bw,	Negative	(NTP, 1997)	
	Micronucleus assay	Mouse peripheral blood cells	Drinking water	3000 - 40000	Negative	(NTP, 1995)	
2-Methylbut-3-en-2-ol [02.123]	Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	500, 1000, 1500 mg/kg	Negative	(BASF, 1992)	Summary in IUCLID data set only. According to this summary, the assay was performed in compliance with GLP and in accordance with OECD guideline 474. One thousand PCEs were counted per animal. The unpublished study report is not available for re-evaluation.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Linalool [02.013])	Micronucleus assay	Mouse bone marrow erythrocytes	Once via gavage	1500 mg/kg	Negative	(Meerts and van de Waart, 2001)	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])	Micronucleus assay	Rat bone marrow cells	Gavage	0, 100, 500 or 1000 mg/kg bw	Negative	(Zamith et al., 1993)	
	Micronucleus assay	Mouse peripheral blood cells	Gavage	Up to 2000 mg/kg bw per day for 13 weeks	Negative	(NTP, 2001)	
(Menthol [02.015])	Host mediated mutation assay	<i>S. typhimurium</i> TA1530 and G46; <i>S. cerevisiae</i> D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Equivocal	(Food and Drug Research Laboratories, Inc., 1975)	Negative results, with exception of the combination <i>S. typhimurium</i> TA1530 – 5000 mg/kg bw and <i>S. cerevisiae</i> D3 – 1150 mg/kg bw per day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels.
	Cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	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.

Table 20: Genotoxicity (*in vivo*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
	Micronucleus assay	B6C3F1 male mouse bone marrow cells	I.P.	0, 250 - 1000 mg/kg bw per 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.
	Dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	Valid.

- (a): 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.
- (b): 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.

ABBREVIATIONS

ADI	Acceptable Daily Intake
bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
CPN	Chronic progressive nephropathy
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EC	European Commission
EFFA	European Flavours and Fragrances Association
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practise
ID	Identity
IOFI	International Organization of the Flavor Industry
Ip	Intraperitoneal
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOAEL	Lowest Observed Adverse Effect Level
MSDI	Maximised Survey-derived Daily Intake

mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA Synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organization