Scientific opinion on flavouring group evaluation 73, Revision 4 (FGE.73Rev4): consideration of alicyclic alcohols, aldehydes, acids and related esters evaluated by JECFA (59th and 63rd meeting) structurally related to primary saturated or unsaturated alicyclic alcohols, aldehydes, acids and esters evaluated by EFSA in FGE.12Rev5

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Scientific Opinion on Flavouring Group Evaluation 73, Revision 4 (FGE.73Rev4): consideration of alicyclic alcohols, aldehydes, acids and related esters evaluated by JECFA (59th and 63rd meeting) structurally related to primary saturated or unsaturated alicyclic alcohols, aldehydes, acids and esters evaluated by EFSA in FGE.12Rev5

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids),
Vittorio Silano, Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Maria Rosaria Milana, Karla Pfaff, Gilles Riviere, Jannavi Srinivasan, Maria de Fátima Tavares Poças, Christina Tlustos, Detlef Wölfle, Holger Zorn, Romualdo Benigni, Leon Brimer, Gerard Mulder, Agneta Oskarsson, Camilla Svendsen, Maria Anastassiadou, Siiri Saarma and Wim Mennes

Abstract

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present revision of FGE.73 concerns the inclusion of four additional flavouring substances (*p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302]) evaluated by JECFA at the 59th meeting. The substances were evaluated through a stepwise approach integrating information on structure–activity relationships, intake from current uses, toxicological thresholds of concern (TTC), and available data on metabolism and toxicity. In agreement with JECFA, the Panel evaluated 22 and one candidate substances via the A and the B-side of the Procedure, respectively, and concluded for all substances 'No safety concern at estimated levels of intake as flavouring substances' based on the maximised survey-derived daily intake (MSDI) approach. The specifications for the materials of commerce have also been considered. Adequate specifications, including complete purity criteria and identity data, are available for 22 out of the 23 JECFA substances evaluated in this FGE. For [FL-no: 09.278], the stereoisomeric composition is not specified. For the six substances with [FL-no: 02.060, 02.091, 09.034, 09.278, 09.302 and 09.712] evaluated in this FGE, use levels have become available and the modified theoretical added maximum daily intakes (mTAMDIs) were estimated. For two substances [FL-no: 09.034, and 09.712], the mTAMDI estimates were above the TTC for their structural class and more detailed information is needed to finalise their evaluation. For the remaining 17 substances evaluated through the Procedure, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment in order to finalise the evaluation.

Keywords: flavourings, *p*-mentha-1,8-dien-7-ol, myrtenol, *p*-mentha-1,8-dien-7-yl acetate, myrtenyl acetate, JECFA, FGE.73

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Table of contents

Abstract 1
1. Introduction 5
1.1. Background and Terms of Reference as provided by the requestor 5
1.1.1. Background 5
1.1.2. Terms of Reference 5
1.2. Interpretation of the Terms of Reference 6
2. Assessment 6
2.1. History of the evaluation of the substances in the present FGE 7
2.2. Presentation of the substances in the JECFA flavouring group 8
2.2.1. Description 8
2.2.2. Isomers 9
2.2.3. Specifications 9
3. Intake estimation 15
3.1. JECFA status 15
3.2. EFSA considerations 15
4. Genotoxicity 15
4.1. Genotoxicity studies – text taken from the JECFA report (JECFA, 2003) 15
4.2. Genotoxicity studies – text taken from EFSA FGE.12Rev4 (EFSA CEF Panel, 2013c) 15
4.3. Genotoxicity studies – text taken from EFSA FGE.209 (EFSA CEF Panel, 2011) 15
4.3.1. *In vitro* data 15
4.3.1.1. Bacterial reverse mutation assay 15
4.3.1.2. Micronucleus assays 16
4.3.2. *In vivo* data 16
4.3.3. Discussion of mutagenicity/genotoxicity data 16
4.3.4. Conclusion on genotoxicity and carcinogenicity 16
4.4. Genotoxicity studies – text taken from EFSA FGE.207 (EFSA CEF Panel, 2013a) 16
4.4.1. *In vitro* data 17
4.4.1.1. Bacterial reverse mutation assay 17
4.4.1.2. Micronucleus assays 17
4.4.2. Conclusion 18
4.5. Genotoxicity studies – text taken from EFSA FGE.213Rev1 (EFSA CEF Panel, 2014a) 19
4.5.1. Bacterial reverse mutation assay 19
4.5.2. Micronucleus assay 19
4.5.3. Conclusion 19
4.6. Genotoxicity studies – text taken from EFSA FGE.208Rev2 (EFSA CEF Panel, 2017) 20
4.6.1. *p*-Mentha-1,8-dien-7-ol [FL-no: 02.060] 20
4.6.1.1. Reverse bacterial mutation assay 20
4.6.1.2. *In vitro* micronucleus assay 20
4.6.2. Myrtenol [FL-no: 02.091] 21
4.6.2.1. Reverse bacterial mutation assay 21
4.6.2.2. *In vitro* micronucleus assay 21
4.6.2.3. BlueScreen™ HC assay 21
4.6.3. *p*-Mentha-1,8-dien-7-yl acetate [FL-no: 09.278] 22
4.6.3.1. Reverse bacterial mutation assay 22
4.6.3.2. *In vitro* micronucleus assay 22
4.6.4. Myrtenyl acetate [FL-no: 09.302] 23
4.6.4.1. Reverse bacterial mutation assay 23
4.6.4.2. *In vitro* micronucleus assay 23
4.6.4.3. BlueScreen™ HC assay 23
4.7. EFSA Considerations 24
5. Application of the Procedure 24
5.1. Application of the Procedure for the safety evaluation to twenty-one alicyclic primary alcohols, aldehydes, acids and related esters, one ester of a phenethyl derivative and one ester of a monocyclic alcohol by JECFA (2002a, 2005b) 24
5.2. Application of the Procedure for the safety evaluation of fifteen primary saturated or unsaturated alicyclic alcohol, aldehyde, and esters by EFSA in FGE.12Rev5 (EFSA CEF Panel, 2014b) 25
5.3. EFSA considerations 25
6. Conclusions 25
Documentation provided to EFSA 26

References 27
Abbreviations 29
Appendix A – Procedure of the safety evaluation 30
Appendix B – Exposure estimate 32
Appendix C – Summary of the genotoxicity data 33
Appendix D – Summary of the safety evaluation 39

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The use of flavourings is regulated under Regulation (EC) No 1334/2008[1] of the European Parliament and Council of 16 December 2008 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 872/2012[2]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

On 27 July 2015, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 208Rev1, (FGE.208Rev1): Consideration of genotoxicity data on representatives for 10 alicyclic aldehydes with the alpha,beta-unsaturation in ring/side-chain and precursors from chemical subgroup 2.2 of FGE.19.

The Panel concluded that *p*-mentha-1,8-dien-7-al [FL-no: 05.117] is genotoxic *in vivo* and as that substance was regarded as the representative of the group, there is a potential safety concern for the other substances in this group. Following this opinion, the Commission withdrew from the Union List of flavourings the representative substance FL-no: 05.117[4] and also the non supported substances 2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde [FL-no: 05.121], myrtenyl formate [FL-no: 09.272], myrtenyl-2-methylbutyrate [FL-no: 09.899] and myrtenyl-3-methylbutyrate [FL-no: 09.900].[5]

On 25 November 2015, the applicant submitted an *in vitro* micronucleus assay and a bacterial reverse mutation assay on the substance myrtenol [FL-no: 02.091]. It also submitted additional studies and data on the other four remaining substances *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL.no: 02.091], myrtenal [FL-no: 05.106], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302] at later stages at the beginning of 2016.

Following also the EFSA opinion of 2015, the Commission amended the conditions of use of these other 5 substances of this group in another Regulation[6] and put a footnote 'under evaluation by EFSA' to them in the Union List of flavourings, pending the evaluation of the additional data.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to evaluate the studies in the submissions on the following five substances of FGE.19 subgroup 2.2: *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL.no: 02.091], myrtenal [FL-no: 05.106], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302], taking into account also the uses reported, the Commission Regulations adopted following the EFSA opinion of July 2015 and any new other safety information relevant available, and, depending on the outcome, proceed to the full evaluation on these 5 flavouring substances, taking into account the requirements of the Commission Regulation (EC) No 1565/2000 and of Regulation (EU) No 1334/2008. The authority is also asked to characterise the hazards and also quantify the exposure also in case its concern on genotoxicity cannot

[1] 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.

[2] 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.

[3] 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.

[4] Commission Regulation (EU) 2015/1760 of 1 October 2015 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of the flavouring substance *p*-mentha-1, 8-dien-7-al. OJ L 257, 2.10.2015, p. 27–29.

[5] Commission Regulation (EU) 2016/637 of 22 April 2016 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of certain flavouring substances. OJ L 108, 23.4.2016, p. 24–27.

[6] Commission Regulation (EU) 2016/1244 of 28 July 2016 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards certain flavouring substances from a group related with an alpha beta unsaturation structure. OJ L 204, 29.7.2016, p. 7–10.

be ruled out and the EFSA CEF panel procedure cannot be applied for any of the substances of the group.

1.2. Interpretation of the Terms of Reference

p-Mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], myrtenal [FL-no: 05.106], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302] were first allocated to FGE.208Rev2 for evaluation with respect to genotoxicity. Based on the new genotoxicity data submitted, the Panel concluded that [FL-no: 02.060, 02.091, 09.278 and 09.302] do not give rise to concern with respect to genotoxicity and can accordingly be evaluated through the Procedure in accordance with Commission Regulation (EC) No 1565/2000. For [FL-no: 05.106], the Panel concluded that the results of the new genotoxicity data were equivocal and not fully adequate to rule out the concern for genotoxicity, therefore presently [FL-no: 05.106] cannot be evaluated through the Procedure. Considering the genotoxicity data from FGE.208Rev2, the four substances evaluated in the current revision of FGE.73, FGE.73Rev4, are [FL-no: 02.060, 02.091, 09.278 and 09.302].

2. Assessment

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000[3], hereafter named the 'EFSA Procedure'. This Procedure is based on the opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995, 1996, 1997, 1999), hereafter named the 'JECFA Procedure'. The CEF Panel compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the 'maximised survey-derived daily intake' (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe.

In its evaluation, JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by JECFA only on the basis of these figures. For substances in the Union List of flavouring substances[7] for which this is the case, the Panel will need the European Union (EU) production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by 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. It is noted that JECFA, at its 65th meeting considered 'how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods' (JECFA, 2006).

In the absence of more accurate 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.

As information on use levels for the flavouring substances has not been requested by JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the

[7] 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.

mTAMDI approach for many of the substances evaluated by JECFA. The Panel will need information on use levels in order to finalise the evaluation.

TTC of 1.5 μg/person per day (step B5) used by JECFA

JECFA uses the toxicological threshold of concern (TTC) of 1.5 μg/person per day as part of the evaluation procedure:

The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 μg/person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the 46th meeting be amended to include the last step on the right-hand side of the original Procedure ('Do the condition of use result in an intake greater than 1.5 μg per day?') (JECFA, 1999).

In line with the opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this TTC of 1.5 μg/person per day.

Genotoxicity

As reflected in the opinion of SCF (1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on, e.g. isomerism.

Structural Relationship

In the consideration of the JECFA-evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding Flavouring Group Evaluation (FGE).

2.1. History of the evaluation of the substances in the present FGE

JECFA has evaluated a group of 26 flavouring substances consisting of alicyclic primary alcohols, aldehydes, acids and related esters (JECFA, 2002a).

In FGE.73, which covered a group of 15 of the 26 JECFA-evaluated substances, the Panel considered that for nine substances [FL-no: 02.114, 02.141, 05.098, 05.112, 08.067, 09.289, 09.488, 09.534 and 09.615] additional data were needed (no European production volumes available, preventing them to be evaluated using the Procedure, and/or missing data on isomerism/composition). For the remaining six of the 15 JECFA-evaluated substances [FL-no: 05.119, 05.123, 08.034, 08.060, 09.028 and 09.536], the Panel agreed with the JECFA conclusion 'no safety concern at estimated levels of intake as flavouring substances' based on the MSDI approach.

The first Revision of FGE.73, FGE.73Rev1, included the assessment of one additional candidate substance, 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104]. No toxicity or metabolism data were provided for the substance. Furthermore, EU production volumes were provided for three substances, [FL-no: 02.141, 09.488 and 09.534] (EFFA, 2010b). After the publication of FGE.73, the following information was received and included in Revision 1: stereoisomeric composition for six substances [FL-no: 02.114, 02.141, 05.098, 08.067, 09.289 and 09.615], and the composition for one substance [FL-no: 05.112] (EFFA, 2010a).

The second Revision of FGE.73, FGE.73Re2, included the assessment of two additional flavouring substances, santalyl acetate [FL-no: 09.034] and santalyl phenylacetate [FL-no: 09.712]. These two substances have been considered with respect to genotoxicity in FGE.207 (EFSA CEF Panel, 2013a) and the Panel concluded that the data available did rule out the concern for genotoxicity and thus concluded that the substances could be evaluated through the Procedure.

Santalyl phenylacetate [FL-no: 09.712] was evaluated by JECFA at its 59th meeting together with other phenethyl substances. With the exception of santanyl phenylacetate [FL-no: 09.712], these phenethyl substances were not α,β-unsaturated substances and were considered by EFSA in FGE.53 with the conclusion 'No safety concern at estimated levels of intake as flavouring substances' based on the MSDI approach. As the phenethyl part of the molecules was considered not to raise concern, the Panel concluded that after santalyl phenylacetate [FL-no: 09.712] was cleared from genotoxic concern in FGE.207, it could be included FGE.73Rev2 together with the other santalyl substance (santalyl acetate [FL-no: 09.034]) from FGE.207.

The third revision of FGE.73 (FGE.73Rev3) concerned the consideration of one JECFA-evaluated substance beta-ionyl acetate [FL-no: 09.305]. beta-Ionyl acetate [FL-no: 09.305] was evaluated by JECFA at its 63rd meeting together with other monocyclic and bicyclic secondary alcohols, ketones and related esters (JECFA, 2005b). beta-Ionyl acetate [FL-no: 09.305] may be hydrolysed to beta-ionol which is considered as a precursor for an α,β-unsaturated ketone, and was originally allocated to and evaluated in FGE.213Rev1 (EFSA CEF Panel, 2014a) in which it was considered not to be of concern with respect to genotoxicity. The Panel concluded that the substance could be included in the FGE.73Rev3.

FGE	Adopted	Link	No. substances
FGE.73	6.3.2008	http://www.efsa.europa.eu/en/efsajournal/pub/868.htm	15
FGE.73Rev1	22.3.2012	http://www.efsa.europa.eu/en/efsajournal/pub/2638.htm	16
FGE.73Rev2	25.9.2013	https://www.efsa.europa.eu/en/efsajournal/pub/3393.htm	18
FGE.73Rev3	24.9.2014	https://www.efsa.europa.eu/en/efsajournal/pub/3862.htm	19
FGE.73Rev4	19.9.2017	http://www.efsa.europa.eu/en/efsajournal/pub/5010.htm	23

FGE: Flavouring Group Evaluation.

The present revision of FGE.73 (FGE.73Rev4) concerns the consideration of four JECFA-evaluated substances *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302]. These substances were originally evaluated in FGE.208Rev2 (EFSA CEF Panel, 2017) in which they were considered to be of no concern with respect to genotoxicity. Therefore, these four substances can be evaluated in the present FGE using the Procedure.

2.2. Presentation of the substances in the JECFA flavouring group

2.2.1. Description

JECFA status

JECFA has at the 59th meeting evaluated a group of 26 flavouring substances consisting of alicyclic primary alcohols, aldehydes, acids and related esters (JECFA, 2002a, 2003).

EFSA considerations

One of the 26 JECFA-evaluated substances is not in the Register [mixture of 2-methyl-5-(2,3-dimethyltricyclo[2.2.1.0(2,6)]hept-3-yl)pent-2-en-1-ol and 2-methyl-5-(2-methyl-3-methylenebicyclo [2.2.1]hept-2-yl)pent-2-en-1-ol] (JECFA-no: 984).

Ten substances [FL-no: 02.060, 02.091, 05.104, 05.106, 05.117, 05.121, 09.034, 09.272, 09.278 and 09.302] are α,β-unsaturated aldehydes or may be metabolised to α,β-unsaturated aldehydes and have been considered together with other α,β-unsaturated aldehydes and ketones. One of these α,β-unsaturated substances, 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104], has been considered with respect to genotoxicity in FGE.209 (EFSA CEF Panel, 2011) and was evaluated through the Procedure in FGE.73Rev1. One additional substance, santalyl acetate [FL-no: 09.034] has been considered with respect to genotoxicity in FGE.207 (EFSA CEF Panel, 2013a), and was evaluated through the Procedure in FGE.73Rev2. The genotoxic properties of the remaining eight of these 10 α, β-unsaturated carbonyl substances were considered together with other α,β-unsaturated aldehydes and ketones in FGE.208 (EFSA CEF Panel, 2013b) for which it was concluded that additional genotoxicity data were required for all eight substances. Among these eight are three substances [FL-no: 05.117, 05.121 and 09.272] that are no longer in the Union List of Flavouring Substances (EFSA CEF Panel, 2015) and these three substances will not be addressed in this FGE.

The Panel also concluded that santalyl phenylacetate [FL-no: 09.712], which is related to santalyl acetate [FL-no 09.034] (already in this FGE), evaluated by JECFA at its 59th meeting together with other phenethyl substances, cleared for genotoxicity concern in FGE.207, should be included in FGE.73Rev2.

Furthermore, the Panel concluded that beta-ionyl acetate [FL-no: 09.305], evaluated by JECFA at its 63rd meeting together with other monocyclic and bicyclic secondary alcohols, ketones and related esters, was cleared for genotoxicity concern in FGE.213Rev1 and should be included in FGE.73Rev3 (JECFA, 2005b).

Four of the initial 26 flavouring substances evaluated by JECFA at its 59th meeting (JECFA, 2002a) viz. *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], *p*-mentha-1,8-dien-7-yl-acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302] were evaluated with respect to genotoxicity in FGE.208Rev2 (EFSA CEF Panel, 2017) and are currently included in FGE.73Rev4.

The Panel concluded that all 23 substances in this FGE are structurally related to the group of primary saturated or unsaturated alicyclic alcohol, aldehyde and esters evaluated by EFSA in the Flavouring Group Evaluation 12, Revision 5 (FGE.12Rev5).

2.2.2. Isomers

JECFA status

Fifteen substances in the group of the JECFA-evaluated substances have one or more chiral centres [FL-no: 02.060, 02.091, 02.114, 02.141, 05.098, 05.119, 05.123, 08.067, 09.034, 09.278, 09.289, 09.302, 09.305, 09.615 and 09.712]. Three substances [FL-no: 09.034, 09.305 and 09.712] can exist as geometrical isomers.

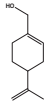
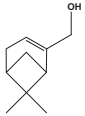
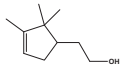
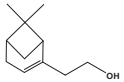
EFSA considerations

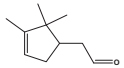
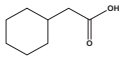
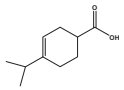
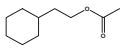
For the two stereoisomeric substances [FL-no: 05.119 and 05.123], the CAS register number (CASrn) is considered to specify the enantiomeric composition (Table 1). [FL-no: 02.060 and 02.091] are reported as racemates. For 15 substances, the information is sufficient. However, for *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278], the stereoisomeric composition of the substance is not specified.

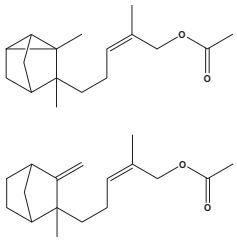
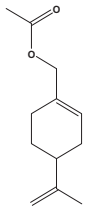
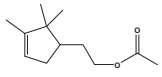
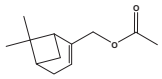
2.2.3. Specifications

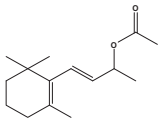
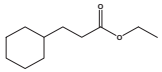
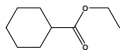
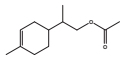
JECFA published specifications for all 23 substances (JECFA, 2002b, 2005a) (see Table 1). For some of these substances, additional information was submitted on behalf of the applicant and Table 1 was updated accordingly.

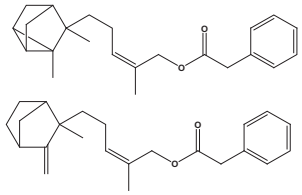
Table 1: Specification summary of the substances in the JECFA flavouring group

FL-no JECFA 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
02.060 974	*p*-Mentha-1,8-dien-7-ol		2,664 2,024 536-59-4	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Miscible	119 (14 hPa) NMR 96%	1.495–1.505 0.956–0.963	Racemate
02.091 981	Myrtenol		3,439 10,285 515-00-4	Liquid $C_{10}H_{16}O$ 152.24	Insoluble Miscible	221 IR NMR 95%	1.490–1.500 0.976–0.983	Racemate
02.114 970	2-(2,2,3-Trimethylcyclopent-3-enyl) ethan-1-ol		3,741 1901-38-8	Liquid $C_{10}H_{18}O$ 154.25	Slightly soluble Miscible	74 (0.8 hPa) NMR 96%	1.470–1.478 0.882–0.894 (20°)	Racemate Synonym (+/–)-campholene alcohol (EFFA, 2010a)
02.141 986	2-(6,6-Dimethylbicyclo[3.1.1] hept-2-en-2-yl)ethan-1-ol		3,938 128-50-7	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Miscible	230 IR NMR 95%	1.490–1.500 0.965–0.973	Racemate (EFFA, 2010a)
05.098 971	*p*-Menth-1-en-9-al		3,178 10,347 29548-14-9	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Miscible	95 (13 hPa) NMR 99%	1.458–1.466 0.904–0.916 (20°)	Racemate (EFFA, 2010a)
05.104 977	2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde		3,389 10,383 116-26-7	Liquid $C_{10}H_{14}O$ 150.22	Insoluble Miscible	70 (1 hPa) NMR 96%	1.525–1.533 0.968–0.980 (20°)	
05.112 978	2,6,6-Trimethylcyclohex-1-en-1-acetaldehyde		3,474 10,338 472-66-2	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Miscible	58 (0.5 hPa) IR NMR 92%	1.480–1.487 0.873–0.885 (20°)	Min. assay (92%) secondary components β-cyclocitral (2-3%), β-ionone (0.5–1%), methyl β-homocyclogeranate (2–4%), ethyl β-homocyclogeranate (0.6–1%) (EFFA, 2010a)

FL-no JECFA 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
05.119 967	2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde		3,592 10,325 4501-58-0	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Miscible	75 (137 hPa) NMR 99%	1.462–1.469 0.918–0.924	CASrn in Register refers to (*R*)-isomer. Register name to be changed to (1*R*) 2,2,3-trimethylcyclopent-3-en- 1-yl acetaldehyde
05.123 968	5-Isopropenyl-2-methylcyclopentanecarboxaldehyde		3,645 55,253-28-6	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Miscible	80 (14 hPa) IR 95%	1.501–1.508 0.940–0.952 (20°)	CASrn in Register refers to (1*R*,2*R*,5*S*)-isomer. Register name to be changed to (1*R*,2*R*,5*S*) 5-isopropenyl-2-methylcyclopentane-carboxaldehyde
08.034 965	Cyclohexylacetic acid		2,347 34 5292-21-7	Solid $C_8H_{14}O_2$ 142.20	Slightly soluble Miscible	242 28–33 NMR 98%	1.459–1.467 1.001–1.009	
08.060 961	Cyclohexanecarboxylic acid		3,531 11,911 98-89-5	Solid $C_7H_{12}O_2$ 128.17	Slightly soluble Miscible	232–233 28–32 IR NMR 98%	1.516–1.520 1.029–1.037	
08.067 976	1,2,5,6-Tetrahydrocuminic acid		3,731 71298-42-5	Solid $C_{10}H_{16}O_2$ 168.24	Slightly soluble Soluble	n.a. 61 NMR 95%	n.a. n.a.	Racemate (EFFA, 2010a)
09.028 964	2-Cyclohexylethyl acetate		2,348 218 21722-83-8	Liquid $C_{10}H_{18}O_2$ 170.25	Insoluble Miscible	211 (996 hPa) NMR 98%	1.442–1.450 0.945–0.948	

FL-no JECFA 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
09.034 985	Santalyl acetate		3,007 224 1323-00-8	Liquid $C_{17}H_{26}O_2$ 262.40	Insoluble Miscible	20.8 (4 hPa) IR 95%	1.485–1.493 0.980–0.986	CASrn in Register refers to incompletely defined substance. '60–65% alpha, 30–35% beta form'. 80–85% *Z* vs 15–20% *E* (for the alpha) and 75–80% *Z* vs 20–25% *E* (for the beta) (EFFA, 2013)
09.278 975	*p*-Mentha-1,8-dien-7-yl acetate		3,561 10,742 15111-96-3	Liquid $C_{12}H_{18}O_2$ 194.27	Insoluble Miscible	218–223 NMR 97%	1.476–1.487 0.972–0.980	Stereoisomeric composition to be specified
09.289 969	alpha-Campholene acetate		3,657 36789-59-0	Liquid $C_{12}H_{20}O_2$ 196.29	Insoluble Miscible	96 (7 hPa) IR NMR 98%	1.453–1.460 0.943–0.949	Commercial product (*S*)-enantiomer (EFFA, 2010a). Register name to be changed to (-)-campholenyl acetate or (*S*)-campholenyl acetate (EFFA, 2010a)
09.302 982	Myrtenyl acetate		3,765 10,887 35670-93-0	Liquid $C_{12}H_{18}O_2$ 194.28	Practically insoluble or insoluble[f] Miscible	134 (49 hPa) IR, NMR, MS 98%	1.470–1.477 0.987–0.996	Racemate (EFFA, 2017) CASrn in the Union List to be changed to 35670-93-0

FL-no JECFA 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
09.305 1409	beta-Ionyl acetate		3,844 10,702 22030-19-9	Liquid $C_{15}H_{24}O_2$ 236.35	Insoluble Soluble	120 (3 hPa) NMR 92%	1.474–1.484 0.934–0.944	Acc. to JECFA: Min. assay value is '92%' and secondary components '2–3% acetic acid; 1–2% beta-ionol'. Racemate, the double bond is mainly *E*-isomer: *E*/*Z* ratio about 50–70%/ 30–50% (EFFA, 2014a)
09.488 966	Ethyl cyclohexanepropionate		2,431 2,095 10094-36-7	Liquid $C_{11}H_{20}O_2$ 184.28	Insoluble Miscible	91 (10 hPa) NMR 98%	1.444–1.452 0.926–0.932	
09.534 963	Ethyl cyclohexanecarboxylate		3,544 11,916 3289-28-9	Liquid $C_9H_{16}O_2$ 156.22	Insoluble Miscible	82 (16 hPa) IR NMR 99%	1.447–1.454 0.966–0.978 (20°)	
09.536 962	Methyl cyclohexanecarboxylate		3,568 11,920 4630-82-4	Liquid $C_8H_{14}O_2$ 142.19	Insoluble Miscible	183 IR NMR 98%	1.439–1.447 0.990–0.999	
09.615 972	*p*-Menth-1-en-9-yl acetate		3,566 10,748 28839-13-6	Liquid $C_{12}H_{20}O_2$ 196.28	Insoluble Miscible	228–232 NMR 97%	1.441–1.448 0.931–0.937	Racemate (EFFA, 2010a)

FL-no JECFA 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
09.712 1022	Santalyl phenylacetate		3,008 239 1323-75-7	Liquid $C_{23}H_{30}O_2$ 338.49	Insoluble Miscible	328 NMR 98%	1.525–1.576 1.022–1.029	CASrn in Register refers to incompletely defined substance. 60–65% alpha-, 30–35% beta- form. 80–85% *Z* vs 15–20% *E* (for the alpha) and 75–80% *Z* vs 20–25% *E* (for the beta) (EFFA, 2013)

Atm: atmosphere (unit); CASrn: CAS register number; CoE: Council of Europe; CoE no: CoE number; EFFA: European Flavour Association; FEMA: Flavor and Extract Manufacturers Association; FEMA no: FEMA number; FL-no: FLAVIS number; ID: Identity; JECFA no: JECFA number; Mol. Formula: Molecular formula; Mol. weight: Molecular weight; Phys. form: Physical form; Refract. index: Refractive index; Spec. gravity: Specific gravity; IR: infrared spectroscopy; NMR: nuclear magnetic resonance; MS: mass spectrometry.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1,013.25 hPa (1 Atm), if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.
(f): No available JECFA specification on solubility in water – see http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/details/en/c/915/

3. Intake estimation

3.1. JECFA status

For all substances evaluated through the JECFA Procedure production volumes (JECFA, 2002a, 2005a, EFFA, 2010b, 2013, 2014a,b, 2016), based on which MSDI values can be calculated, are available for the EU (see Appendix D, Table D.1).

3.2. EFSA considerations

For *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], santalyl acetate [FL-no: 09.034], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278], myrtenyl acetate [FL-no: 09.302] and santalyl phenylacetate [FL-no: 09.712], the flavouring industry submitted normal and maximum use levels (EFFA, 2014b, 2016). Based on these normal use levels, the mTAMDI values were calculated. Flavouring substances with [FL-no: 02.060, 02.091, 09.278 and 09.302] have mTAMDI intake estimates below the TTC for their structural class. Substances with [FL-no: 09.034 and 09.712] have mTAMDI intake estimates above the TTC for their structural class and more reliable data are required to finalise the evaluation.

Use levels and mTAMDI values are presented in Appendix B, Tables B.1 and B.2.

4. Genotoxicity

4.1. Genotoxicity studies – text taken[8] from the JECFA report (JECFA, 2003)

No data on genotoxicity were available for the JECFA-evaluated substances. As these substances are rapidly metabolised *in vivo* to compounds of lower toxicological potential, the Committee concluded that the monocyclic and bicyclic terpenes with alkyl ring substituents and containing an alcohol, aldehyde or carboxylic acid group would have little genotoxic potential *in vivo*.

4.2. Genotoxicity studies – text taken[9] from EFSA FGE.12Rev4 (EFSA CEF Panel, 2013c)

Data are available for the supporting substance 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], but no studies on genotoxicity are available for the 12 candidate substances. The genotoxic potential of the remaining flavouring substances cannot be fully assessed as the data are limited. However, this does not preclude evaluation of the candidate substances in the present group using the Procedure.

4.3. Genotoxicity studies – text taken[9] from EFSA FGE.209 (EFSA CEF Panel, 2011)

The Industry has submitted data concerning genotoxicity studies for 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] (safranal), which is the only substance considered in FGE.209.

4.3.1. *In vitro* data

In vitro genotoxicity assays have been performed on the α,β-unsaturated aldehyde safranal [FL-no: 05.104].

4.3.1.1. Bacterial reverse mutation assay

Safranal has been tested for its ability to induce gene mutations in the bacterial reverse mutation assay according to OECD Guideline 471 (Beevers, 2010) (for details see Appendix C, Table C.1). The concentrations used in the different experiments were based on concentrations observed to give toxic effects in previous experiments. Positive and negative controls were included in all experiments according to current guidelines.

[8] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

[9] The text is taken verbatim from the indicated reference source, but text related to subgroups not included in the present FGE has been removed.

There were some increases in revertant numbers in TA102 in the absence and presence of S9 in the first experiment, but these were of insufficient magnitude to be considered as evidence of mutagenicity, they were not concentration-related, and were not reproducible in the other experiments. In all other strains, there was no evidence of mutagenic activity either in the absence or presence of S9 in any of the experiments.

It is concluded that under the test conditions applied safranal did not induce gene mutations in bacteria.

4.3.1.2. Micronucleus assays

Safranal was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of S9 (Whitwell, 2010). The maximum soluble concentration of 1,250 μg/mL was selected as the maximum concentration for the cytotoxicity range finder test. The concentrations in the main tests were based on toxicity shown in this range finding study (for details, see Appendix C, Table C.1).

At the highest concentration used in the 3 + 21 h treatment in the presence of S9, a small statistical increase in the frequency of micronucleated binucleate cells (MNBN) was observed, but this was set against a low mean concurrent vehicle control response. This concentration induced 62% cytotoxicity, and there was no statistically significant increase in MNBN at the next lowest concentration, which induced 42% cytotoxicity. Therefore, this isolated increase was not considered to be of biological importance. Outside of this isolated observation at a high level of toxicity, no evidence of chromosomal damage or aneuploidy was observed in terms of any increase in the frequency of MNBN in the presence or absence of S9.

It is concluded that under the conditions of this study, safranal did not induce micronuclei in cultured human lymphocytes.

4.3.2. *In vivo* data

Based on the *in vitro* data available, no *in vivo* data are needed.

4.3.3. Discussion of mutagenicity/genotoxicity data

2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] was tested for all three genetic endpoints, gene mutations, structural and numerical chromosomal aberrations. The substance did not induce gene mutations in bacteria and was not clastogenic and/or aneugenic in mammalian cells *in vitro*.

Although this flavouring substance showed evidence of cytotoxicity at high concentrations, it did not induce biologically significant genotoxic responses.

For validation and study results, see Appendix C, Table C.1.

4.3.4. Conclusion on genotoxicity and carcinogenicity

The *in vitro* genotoxicity data on 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] do not indicate genotoxic potential. 2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] was evaluated through the Procedure in FGE.73Rev1.

4.4. Genotoxicity studies – text taken[9] from EFSA FGE.207 (EFSA CEF Panel, 2013a)

The Industry has submitted data concerning genotoxicity studies (EFFA, 2012) for one substance 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] of FGE.19 subgroup 1.1.2 (FGE.201). These data will cover four substances [FL-no: 02.216, 02.217, 09.034 and 09.712] from FGE.19 subgroup 2.1, evaluated in FGE.207.

The new data submitted for 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] covers *in vitro* assays in bacteria and mammalian cell systems.

4.4.1. *In vitro* data

4.4.1.1. Bacterial reverse mutation assay

An Ames assay was conducted in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 to assess the mutagenicity of 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], both in the absence and in the presence of metabolic activation by S9-mix (from livers of rats induced with Aroclor 1254), in three experiments (King, 2000). An initial experiment was carried out in the absence and presence of S9-mix in the five strains, using final concentrations of 2,6-dimethyl-2,5,7-octatriene-1-ol acetate at 5–5,000 μg/plate in the presence of S9-mix activation and 5–1,500 μg/plate in the absence of S9-mix, plus negative (solvent) and positive controls. The standard plate incorporation assay was used. Evidence of toxicity, in terms of a decrease in revertant count, was apparent on all plates treated at 500 μg/plate and above in the absence of S9-mix. In the presence of S9-mix, the test article was toxic at concentrations of 1,500 μg/plate and above for strains TA1537 and TA102, and at 5,000 μg/plate for strains TA98, TA100, and TA1535. In all cases, revertant counts were obtained from at least four different concentrations, and so these data were considered valid for mutation assessment. In the absence of S9-mix activation, no statistically significant increases in revertant numbers were observed in any of the test strains. In the presence of S9-mix activation, no statistically significant increases in revertant numbers were observed for strains TA98, TA100, TA1535 or TA1537, but very small increases in revertant numbers were observed in strain TA102 at 15 and 50 μg/plate which, although statistically significant ($p \leq 0.05$), amounted to only 1.17-fold and 1.18-fold increases over background, respectively. Furthermore, no increases were observed at the higher test concentrations of 150 and 500 μg/plate.

In a second confirmatory experiment using the same conditions, no statistically significant increases in revertant numbers were observed at any concentration in any of the strains, either in the presence or absence of S9-mix activation. To further investigate the potential mutagenic effect in strain TA102 in the presence of S9-mix activation, a third experiment was conducted in that strain only. No statistically significant increases in revertant numbers were observed at any concentration tested.

On this basis, the very small increases seen in only a single experiment at the two lower test concentrations in the presence of S9-mix activation in strain TA102 were not reproducible or concentration-related, and were therefore considered to be chance occurrences and not related to treatment with 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] (King, 2000). It was concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S*. Typhimurium when tested under the conditions of this study. These conditions included treatments at concentrations up to either the limit of toxicity or 5,000 μg/plate (the maximum recommended concentration, according to current regulatory guidelines), in the absence and in the presence of a rat liver metabolic activation system (S9-mix).

4.4.1.2. Micronucleus assays

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] was assayed for the induction of chromosome damage and potential aneugenicity in mammalian cells *in vitro* by examining the effect of compound treatment on the frequency of micronuclei in cultured human peripheral blood lymphocytes (whole blood cultures pooled from two healthy male volunteers in two separate experiments) treated in the absence and presence of a metabolising system (S9-mix) from livers of rats induced with Aroclor 1254 (Whitwell, 2012).

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate was added at 48 h following culture initiation (stimulation by phytohaemagglutinin (PHA)) either for 3 h treatment in the absence or presence of S9-mix plus 21 h recovery, or for 24 h treatment in the absence of S9-mix without recovery. Cytochalasin B (6 μg/mL) was added at the start of the 24-h continuous treatment, or at the start of the 21-h recovery periods following the 3-h treatments, in order to block cytokinesis and generate binucleate cells for analysis. It remained in the cultures until they were harvested 24 h after the start of treatment. A preliminary range-finding experiment had been conducted with and without S9-mix treatment in order to determine the effect of treatment upon Replication Index (RI), which was used as a basis for choosing a range of concentrations to be evaluated in Experiments 1 and 2.

In all of the different treatment conditions and separate experiments, frequencies of MNBN were normal in negative controls and were significantly increased by treatment with the positive control chemical.

In Experiment 1, all three different treatment conditions described above were investigated. In the first treatment condition, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate was added for 3 h in the absence of S9-mix at concentrations of 70, 85, 100 or 120 μg/mL along with positive and negative controls, followed by 21 h recovery. No significant increases in the frequency of MNBN were observed relative to concurrent vehicle controls at any of the concentrations analysed. Furthermore, the MNBN cell frequencies in all treated cultures under this treatment condition fell within the 95th percentile of the normal range.

In the second treatment condition, following 24 h continuous treatment at 20, 40 or 60 μg/mL in the absence of S9-mix without recovery, no increases in the frequency of MNBN cells were obtained that were significantly higher ($p \leq 0.05$) than those observed in concurrent controls. Furthermore, the MNBN cell frequencies in all treated cultures under this treatment condition fell within the 95th percentile of the normal range.

In the third treatment condition, following 3 h treatment with 2,6-dimethyl-2,5,7-octatriene-1-ol acetate at concentrations of 120, 140, 180 or 225 μg/mL in the presence of S9-mix, followed by 21 h recovery, the frequency of MNBN cells were significantly higher ($p \leq 0.05$) than concurrent controls at the top concentration analysed. This concentration induced a 57% mean level of cytotoxicity, which is close to the recommended upper limit for this test procedure. Furthermore, increases in the frequency of MNBN cells were only seen in one replicate (A) where only 394 binucleate cells could be analysed for this test concentration, where cytotoxicity actually exceeded 60%, and where examination of the slides indicated a concentration-related effect on cells without intact cytoplasm. This may have resulted in an underestimation of the cytotoxicity, but it was not observed in the other replicate culture (B).

In Experiment 2, the weak induction of micronuclei that was observed in Experiment 1 in the presence of S9-mix was further investigated. Following treatment for 3 h followed by 21 h recovery in the presence of S9-mix with 2,6-dimethyl-2,5,7-octatriene-1-ol acetate at concentrations of 119.2, 180, 250 or 290 μg/mL, which induced 5%, 19%, 39% and 54% cytotoxicity, respectively, small but statistically significant ($p \leq 0.05$) increases in MNBN cell frequencies were observed at the lowest and highest concentrations analysed. At the highest concentration analysed, only a single replicate culture gave MNBN cell frequencies that exceeded normal historical control values, and it is also noteworthy that the vehicle control frequency was quite low for this particular experiment which might have contributed to the test outcome. Furthermore, additional analysis of spare slides from the replicate cultures at the lowest and highest concentrations analysed resulted in the overall micronucleus frequencies falling within normal ranges. On this basis, the weak statistical significance observed in the first experiment was not reproduced at higher concentrations and similar levels of toxicity, and was therefore not considered to be of biological relevance.

In conclusion, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] was not considered to demonstrate induction of micronuclei in a robust study that achieved required levels of toxicity (Whitwell, 2012).

4.4.2. Conclusion

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] did not induce any biologically significant increases in bacterial mutation when evaluated in an Ames test in the presence and absence of S9 metabolic activation. It did induce weak genotoxic effects in the *in vitro* micronucleus assay in an initial experiment in the presence of S9-mix at the highest concentration only. In a second experiment, although statistically significant increases were observed at the lowest and highest concentrations tested, these increases fell within the historical control range for the testing laboratory, and were not considered to be biologically important. The Panel therefore concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], from subgroup 1.1.2 of FGE.19 (FGE.201), does not give rise to concern with respect to genotoxicity and can accordingly be evaluated through the Procedure. Furthermore, as 2,6-dimethyl-2,5,7-octatriene-1-ol acetate is considered representative for the four precursors for α,β-unsaturated alicyclic aldehydes [FL-no: 02.216, 02.217, 09.034 and 09.712] from subgroup 2.1 of FGE.19 (FGE.207), the genotoxicity concern can also be lifted for these four substances and accordingly they can also be evaluated through the Procedure as well (in FGE.12Rev4 and FGE.73Rev2).

For a summary of *in vitro* genotoxicity data considered by the EFSA in FGE.207, see Appendix C, Table C.2.

4.5. Genotoxicity studies – text taken[9] from EFSA FGE.213Rev1 (EFSA CEF Panel, 2014a)

The substance [FL-no: 09.305] is a precursor of the ketone beta-ionone [FL-no: 07.008] and the conclusion for the precursor has been based in FGE.213Rev1 on the conclusions drawn for the corresponding ketone [FL-no: 07.008].

4.5.1. Bacterial reverse mutation assay

beta-Ionone [FL-no: 07.008] was tested in *S.* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 in the absence and presence of S9-mix (Ballantyne, 2011). In the first experiment, the concentrations were 0.32, 1.6, 8, 40, 200, 1,000 and 5,000 μg/plate of beta-ionone and the plate incorporation methodology was used. Toxicity ranging from slight thinning of the background lawn to complete killing of the tester strains was observed at 1,000 and/or 5,000 μg/plate for all tester strains in the absence and presence of S9-mix. In the second experiment, the concentrations were 10.24, 25.6, 64, 160, 400 and 1,000 μg/plate and the treatments in the presence of S9-mix used the pre-incubation method. Toxicity ranging from thinning of the background lawn and/or reduction in revertant numbers to complete killing of the tester bacteria occurred in all strains at 1,000 μg/plate in the absence and presence of S9-mix and was also seen down to 160 and/or 400 μg/plate for some individual strains. The study design complied with current recommendations and an acceptable top concentration was achieved. There was clearly no evidence of any mutagenic effect induced by beta-ionone in any of the strains, either in the absence or presence of S9-mix.

4.5.2. Micronucleus assay

beta-Ionone [FL-no: 07.008] was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat liver S9-mix fraction as an *in vitro* metabolising system. Cells were stimulated for 48 h with PHA to produce exponentially growing cells, and then treated for 3 h (followed by 21 h recovery) with 0, 30, 50 or 60 μg/mL of beta-ionone in the absence of S9-mix and 0, 80, 100 or 120 μg/mL in the presence of S9-mix. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 52% and 59%, respectively. In a parallel assay, cells were treated for 24 h with 0, 5, 15, and 17.50 μg/mL of beta-ionone in the absence of S9-mix with no recovery period. The top concentration induced 58% cytotoxicity. There were two replicate cultures per treatment and 1,000 binucleate cells per replicate were scored for micronuclei. Thus, the study design complies with current recommendations (OECD Guideline 487), and acceptable levels of cytotoxicity were achieved at the top concentrations used in all parts of the study. Treatment of cells with beta-ionone for 3 h with a 21-h recovery period showed an increase in the frequency of MNBN cells in one single replicate at the concentration of 30 and 120 μg/mL (0.9% and 1.5% respectively) in the absence and presence of S9-mix, respectively. At 30 μg/mL, the lowest concentration tested in the absence of S9-mix, the increase in the frequency of MNBN cells was slightly above the 95% confidence interval of the historical control range (0.2–0.8%). Also in the presence of S9-mix, one replicate of the lowest concentration tested (80 μg/mL) had an increase in the frequency of MNBN cells at the upper limit of the 95% confidence interval of the historical control range (0.10–1.10%) but did not reach statistical significance. To ensure that these single occurrences are random an additional 1,000 binucleate cells were scored from the concurrent controls, 80 and 120 μg/mL cultures. The scoring of further cells resulted in overall mean frequencies of MNBN cells that were not significantly different from concurrent controls and fell below the upper 95% confidence interval of the normal control range (recalculated due to change of stain), and therefore showed that the earlier increases were due to chance. It was concluded that beta-ionone [FL-no: 07.008] did not induce micronuclei up to toxic concentrations when assayed in cultured human peripheral lymphocytes for 3 + 21 h in the absence and presence of S9-mix or when incubated for 24 + 0 h in the absence of S9-mix (Stone, 2011).

4.5.3. Conclusion

The evidence from *in vitro* genotoxicity data for the substance, beta-ionone [FL-no: 07.008] does not indicate a genotoxic potential. Therefore, the substance [FL-no: 09.305] can be evaluated through the Procedure.

For a summary of *in vitro* genotoxicity data considered by the EFSA in FGE.213Rev1, see Appendix C, Table C.3.

4.6. Genotoxicity studies – text taken[9] from EFSA FGE.208Rev2 (EFSA CEF Panel, 2017)

4.6.1. *p*-Mentha-1,8-dien-7-ol [FL-no: 02.060]

4.6.1.1. Reverse bacterial mutation assay

In order to investigate the potential of *p*-mentha-1,8-dien-7-ol [FL-no: 02.060] (purity ≥ 90.3%) and/or its metabolites to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good laboratory practice (GLP) in four strains of *S.* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2*uvr*A, in the presence or absence of metabolic activation in two separate experiments. The test article was evaluated in the initial mutagenicity assay at concentrations of 10, 33.3, 100, 333, 1,000 and 3,333 μg/plate with and without S9-mix, applying the plate incorporation method. Toxicity was observed at 3,333 μg/plate both in the presence and absence of S9-mix in most of the strains, except TA100 and TA1535, showing slightly reduced background at ≥ 1,000 μg/plate, with and without S9-mix. In the confirmatory assay, *p*-mentha-1,8-dien-7-ol was tested at concentrations of 1, 3.33, 10, 33.3, 100, 333, 1,000, 3,333 μg/plate with and without S9-mix, applying the pre-incubation method. Toxicity was observed at concentrations ≥ 333 μg/plate without S9 activation and at concentrations ≥ 1,000 μg/plate in the presence of S9 activation. No precipitate was observed at any tested concentration in any tester strain with or without S9-mix. Appropriate positive control chemicals and dimethyl sulfoxide (DMSO, as vehicle control) were evaluated concurrently and all test and control articles were evaluated in triplicate plates. All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while negative controls were within the historical control ranges. No increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains with or without S9-mix (Wagner, 2016).

The Panel considered the results of this assay as negative.

4.6.1.2. *In vitro* micronucleus assay

The *in vitro* micronucleus assay was carried out according to OECD Test Guideline 487 (OECD, 2014) and following GLP. Human peripheral blood lymphocytes from healthy donors, stimulated with PHA, were treated with *p*-mentha-1,8-dien-7-ol [FL-no: 02.060] (purity ≥ 90.3%) (Roy, 2016) in a dose-range finding assay performed at concentrations ranging from 1 to 1,520 μg/mL for 4 h with and without S9-mix and 24 h without S9-mix. At the termination of the treatment period, precipitate and haemolysis were observed at concentrations ≥ 1,000 μg/mL and ≥ 400 μg/mL, respectively, in all three treatment conditions.

Based on the dose-range finding results, duplicate cultures of lymphocytes were treated with the test article 44–48 h after culture initiation at concentrations ranging from 100 to 375 μg/mL for 4 h with and without S9-mix.

Cytochalasin B (final concentration of 6 μg/mL) was added to each culture after the 4 h treatment period, while in the 24 h treatment cultures were treated with the test article in the presence of cytochalasin B.

Appropriate vehicle (DMSO) and positive controls were used (mitomycin C and vinblastine in the absence of S9-mix, cyclophosphamide in the presence of S9-mix). All positive control compounds induced a statistically significant increase of micronucleus (MN) frequency and the system was considered sensitive and valid.

Two thousand cells were scored per concentration. Based on the level of cytotoxicity observed, three concentration levels were selected for MN analysis in each experimental condition: (i) 25, 50 and 100 μg/mL, 24 h treatment (16%, 31% and 58% cytotoxicity, respectively); (ii) 100, 250 and 325 μg/mL, 4 h treatment without S9-mix (16%, 24% and 58% cytotoxicity, respectively); (iii) 100, 225 and 275 μg/mL, 4 h treatment with S9-mix (3%, 18% and 51% cytotoxicity, respectively). No statistically significant increase in the frequency of micronuclei was observed after treatment with the test article at any concentration analysed (Roy, 2016).

The Panel considered the results of this assay as negative.

4.6.2. Myrtenol [FL-no: 02.091]

4.6.2.1. Reverse bacterial mutation assay

In order to investigate the potential of myrtenol (purity ≥ 97%) and/or its metabolites to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline 471 (OECD, 1997) and following GLP in four strains of *S.* Typhimurium (TA98, TA100, TA1535 and TA1537) and *E. coli* WP2*uvr*A, in the presence or absence of metabolic activation applying the plate incorporation method. The test article was evaluated in the initial mutagenicity assay at concentrations of 5, 16, 50, 160, 500, 1,600, and 5,000 μg/plate with and without S9-mix. A confirmatory assay was subsequently performed at concentrations of 16, 50, 160, 500, 1,600 and 5,000 μg/plate with and without S9-mix. Appropriate positive control chemicals and DMSO (as vehicle control) were evaluated concurrently, and all test and control articles were evaluated in triplicate plates. All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while negative controls were within the historical control ranges. No precipitate was observed at any tested concentration in any tester strain with or without S9-mix. Toxicity, as evident by the absence or reduction in the mean number of revertant colonies and absence or reduction in the background bacterial lawn, was observed in both experiments at 5,000 μg/plate in all tester strains with and without S9-mix except WP2*uvr*A, where toxicity was observed at concentrations ≥ 1,600 μg/plate without S9-mix. No increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains with or without S9-mix (Bhalli and Phil, 2015a).

The Panel considered the results of this assay as negative.

4.6.2.2. *In vitro* micronucleus assay

The *in vitro* micronucleus assay was carried out according to OECD Test Guideline 487 (OECD, 2010) and following GLP. Human peripheral blood lymphocytes from healthy donors, stimulated with PHA, were treated with myrtenol (purity ≥ 97%) in a dose-range finding assay performed in single cultures at concentrations ranging from 28.2 to 1,000 μg/mL for 3 h with and without S9-mix and 24 h without S9-mix. No precipitate was observed at the end of treatment and/or harvest at any tested concentration in any treatment condition. In the 3 h treatments, haemolysis was observed at 1,000 μg/mL at the end of treatment.

Based on the dose-range finding results, duplicate cultures of lymphocytes were treated with the test article 48 h after culture initiation at concentrations ranging from 15.3 to 80.0 μg/mL in the 24 h treatment. The test article was also evaluated in the 3 h treatments at 224–500 μg/mL with and without S9-mix.

Cytochalasin B (final concentration of 6 μg/mL) was added to each culture after the 3 h treatment period, while in the 24 h treatment cultures were treated with the test article in the presence of cytochalasin B. Appropriate vehicle (DMSO) and positive controls were used (mitomycin C in the absence of S9-mix, cyclophosphamide in the presence of S9-mix). All positive control compounds induced a statistically significant increase of MN frequency and the system was considered sensitive and valid.

Two thousand cells were scored per concentration. Based on the level of cytotoxicity observed, at least three concentration levels were selected for MN analysis in each experimental condition: (i) 30.6, 47.2 and 52.5 μg/mL with the 24 h treatment (26%, 41% and 54% cytotoxicity, respectively); (ii) 407, 451 and 475 μg/mL with the 3 h treatment with S9-mix (15%, 34% and 46% cytotoxicity, respectively); (iii) 368, 387, 451 and 475 μg/mL with the 3 h treatment without S9-mix (19%, 35%, 43% and 64% cytotoxicity, respectively). No statistically significant increase in the frequency of micronuclei was observed after treatment with the test article at any concentration analysed compared to the respective concurrent vehicle controls (Bhalli and Phil, 2015b).

The Panel considered the results of this assay as negative.

4.6.2.3. BlueScreen™ HC assay

Myrtenol [FL-no: 02.091] was tested in a BlueScreen™ HC assay for cytotoxicity and genotoxicity using a genetically modified strain of cultured human lymphoblastoid TK6 cells, both in the presence and absence of metabolic activation. The study authors concluded that myrtenol did not induce genotoxicity at the concentrations tested (Birrell, 2013a).

4.6.3. *p*-Mentha-1,8-dien-7-yl acetate [FL-no: 09.278]

4.6.3.1. Reverse bacterial mutation assay

In order to investigate the potential of *p*-mentha-1,8-dien-7-yl acetate (purity 96.5%) and/or its metabolites to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline 471 (OECD, 1997) and following GLP in five strains of *S.* Typhimurium (TA98, TA100, TA1535, TA1537 and TA102), in the presence or absence of metabolic activation, in two separate experiments. In the first experiment, *p*-mentha-1,8-dien-7-yl acetate was tested at concentrations of 5, 16, 50, 160, 500, 1,600, and 5,000 μg/plate with and without S9-mix, applying the plate incorporation assay. In the second experiment, *p*-mentha-1,8-dien-7-yl acetate was tested at concentrations of 1.6, 5, 16, 50, 160, 500 and 1,600 μg/plate with and without S9-mix, applying the pre-incubation method. Appropriate positive control chemicals and DMSO (as vehicle control) were evaluated concurrently. All test and positive control articles were evaluated in triplicate plates; the vehicle control was evaluated in quintuplicate.

All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while negative controls were within the historical control ranges.

No precipitate was observed at any tested concentration in any tester strain with or without S9-mix.

In the first experiment, toxicity, as evident by the absence or reduction in the mean number of revertant colonies and absence or reduction in the background bacterial lawn, was observed at 500 μg/plate and above in all tester strains in the absence of S9-mix and for strain TA1537 in the presence of S9-mix. For all other strains, in the presence of S9-mix, toxicity was observed at concentrations above 1,600 μg/plate.

In the second experiment, toxicity was observed at 500 μg/plate and above in all strains in the absence of S9-mix and in strain TA1535 and TA102 in the presence of S9-mix. Toxicity was observed at 160 μg/plate and above in strains TA98, TA100 and TA1537 in the presence of S9-mix.

No increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains with or without S9-mix (Lloyd, 2016a).

The Panel considered the results of this assay as negative.

4.6.3.2. *In vitro* micronucleus assay

The *in vitro* micronucleus assay was carried out according to OECD Test Guideline 487 (OECD, 2014) and following GLP. Human peripheral blood lymphocytes from healthy donors, stimulated with PHA, were treated with *p*-mentha-1,8-dien-7-yl acetate (purity 96.5%) in a dose-range finding assay performed in single cultures at concentrations ranging from 7.05 to 1,943 μg/mL for 3 h with and without S9-mix and 24 h without S9-mix. At the time of treatment, precipitate was observed at concentrations ≥ 151.1 μg/mL in all three treatment conditions.

Based on the dose-range finding results, duplicate cultures of lymphocytes were treated with the test article 48 h after culture initiation at concentrations ranging from 25 to 250 μg/mL for treatments at 3 + 21 h without metabolic activation. Concentrations ranging from 50 to 500 μg/mL were tested in the treatment at 3 + 21 h with metabolic activation. Concentrations ranging from 10 to 150 μg/mL were tested in the treatment at 24 h without metabolic activation. Cytochalasin B (final concentration of 6 μg/mL) was added to each culture at the time of treatment. Appropriate vehicle (DMSO) and positive controls were used (mitomycin C and noscapine in the absence of S9-mix, cyclophosphamide in the presence of S9-mix). All positive control compounds induced a statistically significant increase of MN frequency and the system was considered sensitive and valid. Two thousand cells were scored per concentration. Based on the level of cytotoxicity observed, at least three concentration levels were selected for MN analysis in each experimental condition: (i) 30, 60 and 80 μg/mL with the 24 h treatment (12%, 39% and 55% cytotoxicity, respectively); (ii) 150, 240 and 280 μg/mL with the 3 h treatment with S9-mix (11%, 41% and 55% cytotoxicity, respectively); iii) 100, 120 and 130 μg/mL with the 3 h treatment without S9-mix (9%, 44% and 67% cytotoxicity, respectively). In the treatment of 3 + 21 h with S9-mix, precipitate was observed at 150 μg/mL and above. In the treatment of 3 + 21 h without S9-mix, precipitate was observed at 120 and 130 μg/mL. *p*-Mentha-1,8-dien-7-yl acetate did not induce a statistically significant increase of MNBN cells at any concentration analysed (Lloyd, 2016b).

The Panel considered the results of this assay as negative.

4.6.4. Myrtenyl acetate [FL-no: 09.302]

4.6.4.1. Reverse bacterial mutation assay

In order to investigate the potential of myrtenyl acetate (purity 97.6%) and/or its metabolites to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline 471 (OECD, 1997) and GLP in five strains of *S.* Typhimurium (TA98, TA100, TA1535, TA1537 and TA102), in the presence or absence of metabolic activation, in two separate experiments. In the first experiment, myrtenyl acetate was tested at concentrations of 5, 16, 50, 160, 500, 1,600 and 5,000 μg/plate with and without S9-mix, applying the plate incorporation assay. In the second experiment, myrtenyl acetate was tested at concentrations of 3.28, 8.2, 20.5, 51.2, 128, 320, 800 and 2,000 μg/plate with and without S9-mix, applying the pre-incubation method. Appropriate positive control chemicals and DMSO (as vehicle control) were evaluated concurrently. All test and positive control articles were evaluated in triplicate plates; the vehicle control was evaluated in quintuplicate.

All positive control chemicals induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while negative controls were within the historical control ranges.

No precipitate was observed at any tested concentration in any tester strain with or without S9-mix.

In the first experiment, toxicity, as evident by the absence or reduction in the mean number of revertant colonies and absence or reduction in the background bacterial lawn, was observed in both experiments at 500 μg/plate and above in all tester strains in the absence of S9-mix and strain TA1535 in the presence of S9-mix. For the other four strains, in the presence of metabolic activation, toxicity was observed at 1,600 μg/plate and above.

In the second experiment, toxicity was observed at 320 μg/plate and above in strains TA98, TA100, TA1535 and TA1537 in the presence and absence of S9-mix. In strain TA102, toxicity was observed at 2,000 μg/plate in the presence and absence of S9-mix.

No increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains with or without S9-mix (Mc Garry, 2016a).

The Panel considered the results of this assay as negative.

4.6.4.2. *In vitro* micronucleus assay

The *in vitro* micronucleus assay was carried out according to OECD Test Guideline 487 (OECD, 2014) and following GLP. Human peripheral blood lymphocytes from healthy donors, stimulated with PHA, were treated with myrtenyl acetate (purity 97.6%) in a dose-range finding assay performed in single cultures at concentrations ranging from 7.05 to 1,943 μg/mL for 3 h with and without S9-mix and 24 h without S9-mix. At the time of treatment, precipitate was observed at concentrations ≥ 250 μg/mL.

Based on the dose-range finding results, duplicate cultures of lymphocytes were treated with the test article 48 h after culture initiation at concentrations ranging from 5 to 200 μg/mL for treatments without metabolic activation. Concentrations ranging from 25 to 500 μg/mL were tested in the treatment at 3 + 21 h with metabolic activation. Cytochalasin B (final concentration of 6 μg/mL) was added to each culture after the 3-h treatment period, while in the 24-h treatment cultures were treated with the test article in the presence of cytochalasin B. Appropriate vehicle (DMSO) and positive controls were used (mitomycin C and noscapine in the absence of S9-mix, cyclophosphamide in the presence of S9-mix). All positive control compounds induced a statistically significant increase of MN frequency and the system was considered sensitive and valid. Two thousand cells were scored per concentration. Based on the level of cytotoxicity observed, at least three concentration levels were selected for MN analysis in each experimental condition: (i) 10, 20 and 40 μg/mL with the 24 h treatment (2%, 21% and 52% cytotoxicity, respectively); (ii) 150, 250 and 325 μg/mL with the 3 h treatment with S9-mix (7%, 36% and 53% cytotoxicity, respectively); (iii) 20, 60, 80 and 90 μg/mL with the 3 h treatment without S9-mix (0%, 12%, 45% and 48% cytotoxicity, respectively). No statistically significant increase in the frequency of micronuclei was observed after treatment with the test article at any concentration analysed (Mc Garry, 2016b).

The Panel considered the results of this assay as negative.

4.6.4.3. BlueScreen™ HC assay

Myrtenyl acetate [FL-no: 09.302] was tested in a BlueScreen™ HC assay for cytotoxicity and genotoxicity using a genetically modified strain of cultured human lymphoblastoid TK6 cells, both in the presence and absence of metabolic activation. The study authors concluded that myrtenyl acetate did not induce genotoxicity at the concentrations tested (Birrell, 2013b).

4.7. EFSA Considerations

The present revision of FGE.73, FGE.73Rev4, contains 23 substances and includes the assessment of four additional flavouring substances, *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302]. These substances have structural alerts for genotoxicity, but this concern has been alleviated as described in FGE.208Rev2 (EFSA CEF Panel, 2017), where the Panel concluded that *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], myrtenyl acetate [FL-no: 09.302] and *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] do not give rise to concern with respect to genotoxicity. Therefore, *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], myrtenyl acetate [FL-no: 09.302] and *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] can be evaluated through the Procedure in this FGE.73Rev4. In revision 3 of FGE.73, beta-ionyl acetate [FL-no: 09.305] was evaluated through the Procedure. In revision 2 of FGE.73, santalyl acetate [FL-no: 09.034] and santalyl phenylacetate [FL-no: 09.712] were evaluated through the Procedure, due to that concern for genotoxicity for these two substances had been evaluated and ruled out in FGE.207. No genotoxicity data are available for the remaining 16 JECFA evaluated substances. However, this will not preclude the evaluation of these substances using the Procedure as has been done by JECFA.

5. Application of the Procedure

5.1. Application of the Procedure for the safety evaluation to twenty-one alicyclic primary alcohols, aldehydes, acids and related esters, one ester of a phenethyl derivative and one ester of a monocyclic alcohol by JECFA (2002a, 2005b)

According to JECFA, all 23 substances belong to structural class I using the decision tree approach presented by Cramer et al. (1978).

JECFA concluded for 20 of the alicyclic primary alcohols, aldehydes, acids and related esters, and for santalyl phenylacetate [FL-no: 09.712], an ester of the phenethyl derivatives, and for beta-ionyl acetate [FL-no: 09.305] at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the TTC for their structural class I (step A3).

JECFA concluded for 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] (safranal) at step B4 in the JECFA Procedure – i.e. the substance cannot be expected to be metabolised to innocuous products (step 2) and an adequate no observed adverse effect level (NOAEL) exists to provide a margin of safety (step B4). This evaluation was reached by the following procedure: step B3. The daily per capita intake of the monocyclic substance with two endocyclic double-bonds evaluated at this step, 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104], was below the TTC for daily human intake of compounds of structural class I, and its evaluation therefore proceeded to step B4.

Step B4. As the agent evaluated at this step, 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] (safranal), is structurally related to perillyl alcohol [FL-no: 02.060], data on the toxicity of perillyl alcohol were used to evaluate its safety. Perillyl alcohol given by intragastric gavage changed the weights of several organs in female rats when given at 400 mg/kg body weight (bw) per day, but not at 120 mg/kg bw per day, in a 90-day study; changes in organ weights were not reported in male rats. Doses of 40, 120 and 400 mg/kg bw per day produced hyperexcitability and salivation, which the authors considered may have been due to its irritating properties (NCI, 1996). A daily dose of 120 mg/kg bw was well tolerated by dogs in a 90-day study (NCI, 1996). The daily intake of 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] (safranal) is 0.058 μg/kg bw in Europe and 0.001 μg/kg bw in the USA. The margin of safety between these intakes and 120 mg/kg bw per day is > 2,000,000. The compound also shares structural similarities with alpha-ionone and beta-ionone [FL-no: 07.007] and [FL-no: 07.008], which were evaluated by the Committee at its fifty-first meeting (JECFA, 2000). The no-observed-effect levels (NOELs) for these compounds were 10 mg/kg bw per day in a 90-day study in rats, providing a margin of safety of about 200,000. Therefore, 2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde [FL-no: 05.104] (safranal) would not be a safety concern.

In conclusion, JECFA evaluated all 23 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 23 alicyclic alcohols, aldehydes, acids and related esters are summarised in Appendix D, Table D.1.

5.2. Application of the Procedure for the safety evaluation of fifteen primary saturated or unsaturated alicyclic alcohol, aldehyde, and esters by EFSA in FGE.12Rev5 (EFSA CEF Panel, 2014b)

Fifteen candidate substances were evaluated in FGE.12Rev5. All 15 substances were classified into structural class I, using the decision tree approach presented by Cramer et al. (1978).

It was anticipated that all 15 substances will be metabolised to innocuous products at the estimated levels of intake and accordingly proceed via the A-side of the Procedure. The estimated daily per capita intakes of the 15 substances range from 0.011 to 100 μg, which is below the TTC of 1,800 μg/person per day for structural class I.

The Panel concluded all substances in FGE.12Rev5 at step A3 as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The stepwise evaluations of the 15 substances are summarised in Table D.2.

5.3. EFSA considerations

The Panel agrees with the application of the Procedure as performed by JECFA for the 23 substances in the groups of alicyclic alcohols, aldehydes, acids and related esters.

The Panel noted that each one of the three substances [FL-no: 02.060, 05.123 and 09.278] has a terminal double bond. Although theoretically the double bond may be oxidised to give reactive epoxides, for these substances the metabolism via this pathway is not expected, since the terminal double bond is present in molecules that have an additional functional group at the end distal from each one of the double bonds. The alcohol and aldehyde functions of [FL-no: 02.060 and 05.123] are expected to be readily attacked by oxidation processes and the ester function of [FL-no: 09.278] can be expected to be hydrolysed, ultimately yielding the corresponding carboxylic acids. Biochemical attack of these carboxylic acids via, e.g. conjugation with glucuronic acid is expected to be much more efficient and rapid than microsomal oxidation.

6. Conclusions

The current revision of FGE.73Rev4 considers in total 23 substances evaluated by JECFA at its 59th and 63rd meetings. Of these, 19 substances have already been evaluated in previous versions of this FGE. The present revision includes four additional flavouring substances evaluated by JECFA at the 59th meeting: *p*-mentha-1,8-dien-7-ol [FL-no: 02.060], myrtenol [FL-no: 02.091], *p*-mentha-1,8-dien-7-yl acetate [FL-no: 09.278] and myrtenyl acetate [FL-no: 09.302].

The Panel concluded that the 23 substances are structurally related to the group of 15 primary saturated or unsaturated alicyclic alcohol, aldehyde, and esters evaluated by EFSA in the Flavouring Group Evaluation 12, Revision 5 (FGE.12Rev5).

The Panel agrees with the application of the Procedure as performed by JECFA for the substances considered in this FGE.

For all 23 JECFA evaluated alicyclic alcohols, aldehydes, acids and related esters evaluated [FL-no: 02.060, 02.091, 02.114, 02.141, 05.098, 05.104, 05.112, 05.119, 05.123, 08.034, 08.060, 08.067, 09.028, 09.034, 09.278, 09.289, 09.302, 09.305, 09.488, 09.534, 09.536, 09.615 and 09.712], the Panel agreed with JECFA conclusion that, according to the Procedure, they are not expected to be of safety concern when used as flavouring substances based on the MSDI approach.

In order to determine whether the conclusion for the JECFA-evaluated 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 data, are available for 22 out of the 23 JECFA substances evaluated in this FGE. For [FL-no: 09.278] the stereoisomeric composition is not specified.

Only for six substances with [FL-no: 02.060, 02.091, 09.034, 09.278, 09.302 and 09.712] evaluated in this FGE, normal and maximum use levels have become available. Based on these normal use levels, mTAMDI values can be calculated. Flavouring substances with [FL-no: 02.060, 02.091, 09.278 and 09.302] have mTAMDI intake estimates below the TTC for their structural class. The Panel noted that these four substances are evaluated via the A-side of the Procedure. Substances with [FL-no: 09.034 and 09.712] have mTAMDI intake estimates above the TTC for their structural class and more reliable data are required to finalise the evaluation. For the remaining 17 substances evaluated through the

Procedure, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment in order to finalise the evaluation.

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Abbreviations

bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DMSO	dimethyl sulfoxide
EFFA	European Flavour Association
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practice
ID	Identity
IR	infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MN	micronucleus
MNBN	micronucleated Binucleate cells
MS	mass spectrometry
MSDI	maximised survey-derived daily intake
mTAMDI	modified theoretical added maximum daily intake
NMR	nuclear Magnetic Resonance
No	Number
NOAEL	no observed adverse effect level
NOEL	no-observed-effect level
OECD	Organisation for Economic Cooperation and Development
PHA	phytohaemagglutinin
RI	Replication Index
SCF	Scientific Committee on Food
TTC	Toxicological Threshold of Concern
WHO	World Health Organization

Appendix A – Procedure of the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, 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, 1996, 1997, 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 and III) for which toxicological thresholds of concern (TTCs) (human exposure thresholds) have been specified. Exposures below these TTCs 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 TTCs for these structural classes of 1,800, 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 products[10] (step 2)?
- Do their exposures exceed the TTC for the structural class (steps A3 and B3)?
- Are the flavourings or their metabolites endogenous[11] (step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (steps 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.

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

[11] '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, 1997).

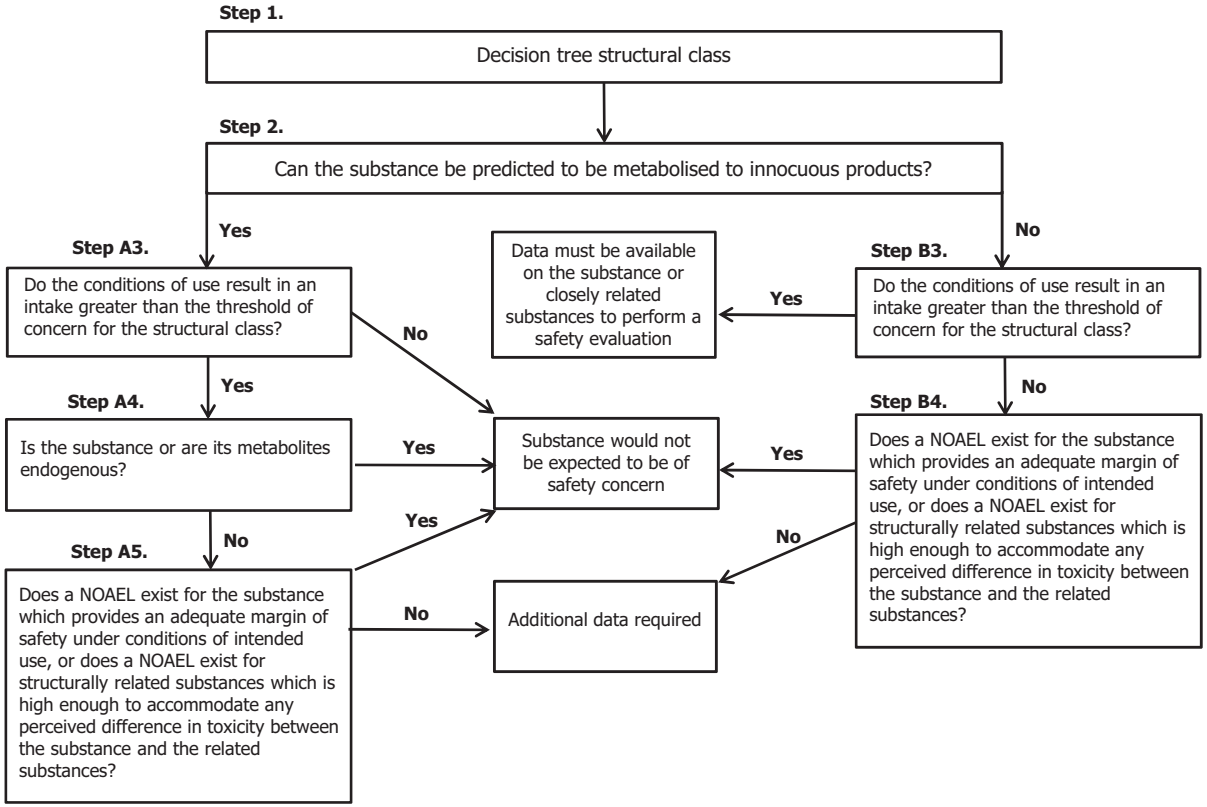


Figure A.1: Procedure for the safety evaluation of chemically defined flavouring substances

Appendix B – Exposure estimate

Table B.1: Normal and maximum use levels (mg/kg food) for the JECFA-evaluated substances in FGE.73Rev4 (EFFA, 2014b, 2016)

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**
02.060	5	–	5	5	–	5	5	8	–	–	–	–	–	–	1	0.5	–	2
	10	–	10	10	–	25	10	50	–	–	–	–	–	–	5	1	–	10
02.091	5	–	5	–	–	5	5	–	–	–	–	–	–	–	0.5	0.5	0.1	0.1
	10	–	10	–	–	10	10	–	–	–	–	–	–	–	1	1	1	1
09.034	7	5	10	7	–	10	5	10	2	2	2	2	5	10	5	10	20	5
	35	25	50	35	–	50	25	50	10	10	10	10	25	50	25	50	100	25
09.278	5	–	5	–	–	5	5	7.2	1	–	–	–	–	–	0.5	0.5	–	2
	10	–	10	–	–	10	16	16	3	–	–	–	–	–	2	2	–	10
09.302	5	–	5	5	–	5	5	2	–	–	–	–	–	–	1	2.4	–	1
	10	–	10	10	–	30	10	5	–	–	–	–	–	–	4	4	–	20
09.712	7	5	10	7	–	10	5	10	2	2	2	2	5	10	5	10	20	5
	35	25	50	35	–	50	25	50	10	10	10	10	25	50	25	50	100	25

Table B.2: Estimated intakes based on the MSDI approach and the mTAMDI approach (EFFA, 2014b, 2016)

FL-no	EU register name	MSDI – EU (μg/capita per day)	mTAMDI (μg/person per day)	Structural class	Toxicological threshold of concern (μg/person per day)
02.060	*p*-Mentha-1,8-dien-7-ol	0.34	1,500	Class I	1,800
02.091	Myrtenol	0.1	980	Class I	1,800
09.034	Santalyl acetate	0.1	3,900	Class I	1,800
09.278	*p*-Mentha-1,8-dien-7-yl acetate	1.4	1,300	Class I	1,800
09.302	Myrtenyl acetate	0.91	1,200	Class I	1,800
09.712	Santalyl phenylacetate	0.029	3,900	Class I	1,800

MSDI: maximised survey-derived daily intake; mTAMDI: modified theoretical added maximum daily intake; ND: not derived.

Appendix C – Summary of the genotoxicity data

Table C.1: Genotoxicity data (*in vitro*) EFSA/FGE.209 (EFSA CEF Panel, 2011)

Chemical name FL-no	Test system *in vitro*	Test object	Concentrations of test substance	Result	Reference	Comments
2,6,6-Trimethylcyclohexa-1, 3-diene-1-carbaldehyde [05.104]	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537 and TA102	1.6, 8, 40, 200, 1,000, 5,000 μg/plate	Negative(d)	Beevers (2010)	Valid study. First experiment: Standard plate ± S9. Toxicity was observed in all strains with and without S9 at 5,000 μg/plate and in TA1537 and TA102 with S9 at 1,000 μg/plate
		S. Typhimurium TA98, TA100, TA1535, TA1537 and TA102	125, 250, 500, 1,000, 2,000, 5,000 μg/plate	Negative(d)	Beevers (2010)	Valid study. Second experiment: Standard plate without S9. Toxicity was observed at 2,000 μg/plate and above
		S. Typhimurium TA98, TA100 and TA1535	62.5, 125, 250, 500, 1,000, 2,000, 5,000 μg/plate	Negative(d)	Beevers (2010)	Valid study. Second experiment with S9 and pre-incubation: Toxicity was observed at 500 μg/plate and above
		S. Typhimurium TA1537 and TA102	62.5, 125, 250, 500, 1,000, 2,000 μg/plate	Negative(d)	Beevers (2010)	Valid study. Second experiment with S9 and pre-incubation: Toxicity was observed at 500 μg/plate and above,.
		S. Typhimurium TA98, TA100, TA1535, TA1537 and TA102	15.625, 31.25, 62.5, 125, 250, 500 μg/plate	Negative(d)	Beevers (2010)	Valid study. Third experiment with S9 and pre-incubation: Toxicity was observed at 250 μg/plate and above
	Micronucleus induction	Human peripheral blood lymphocytes	0, 40, 60, 90 μg/mL(a)	Negative(e)	Whitwell (2010)	Valid study
			0, 80, 100, 120, 140 μg/mL(b)			
			0, 4, 8, 12 μg/mL(c)			

(a): 3 h treatment with 21 h recovery without S9.
(b): 3 h treatment with 21 h recovery with S9.
(c): 24 h treatment with no recovery without S9.
(d): The assays were performed according to OECD Guideline 471 and in compliance with GLP.
(e): This assay is performed in accordance with OECD Guideline 487.

Table C.2: Summary of additional genotoxicity data for [FL-no: 09.931] of subgroup 1.1.2 used in FGE.207 (EFSA CEF Panel, 2013a)

Chemical name [FL-no:]	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [09.931]	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537 and TA102	5–1,500 μg/plate[(a),(c)] 5–5,000 μg/plate[(b),(c)]	Negative[(a),(c)] Equivocal	King (2000)	Reliable without restriction. GLP study in compliance with OECD Guideline 471. A small increase in TA102 revertant numbers was seen at 15 and 50 μg/plate in the presence of S9-mix, but not at higher concentrations
		S. Typhimurium TA98, TA100, TA1535, TA1537 and TA102	5–1,500 μg/plate[(a),(c)] 5–5,000 μg/plate[(b),(c)]	Negative[(a),(c)] Negative[(b),(c)]		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 μg/plate in the presence of S9-mix was not reproduced in the second experiment
		S. Typhimurium TA102	5–1,500 μg/plate[(b),(c)]	Negative		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 μg/plate in the presence of S9-mix was not reproduced in the third experiment
	Micronucleus assay	Human peripheral blood lymphocytes (Male Donors)	70–120 μg/mL[(a),(d)] 120–225 μg/mL[(b),(d)] 20–60 μg/mL[(a),(e)] 119.2–290 μg/mL[(b),(d)]	Weak positive +S9; Re-test within normal values	Whitwell (2012)	Reliable without restriction. GLP study in compliance with OECD Guideline 487. Weak evidence of inducing micronuclei in the presence of S9-mix in a first experiment (increases only in one culture). A retest under the same conditions and using a higher top concentration resulted in MNBN frequencies within the historical negative control range at 95th percentile, but were statistically significant due to low vehicle control values

GLP: Good laboratory practice; OECD: Organisation for Economic Cooperation and Development; MNBN: micronucleated binucleate cells.
(a): Without S9-mix metabolic activation.
(b): With S9-mix metabolic activation.
(c): Plate incorporation method.
(d): 3-h incubation with 21-h recovery period.
(e): 24-h incubation with no recovery period.

Table C.3: Genotoxicity data (*in vitro*) EFSA/FGE.213Rev1 (EFSA CEF Panel, 2014a)

Chemical name [FL-no:]	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
β-Ionone [07.008]	Gene mutation (preincubation)	*S.* Typhimurium TA98, TA100, TA1535, TA1537	1–180 μg/plate	Negative [(a)]	Mortelmans et al. (1986)	**Valid**
	Gene mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537	3 mmol/plate	Negative[(a)]	Florin et al. (1980)	**Insufficient validity (spot test, not according to OECD Guideline, methods and results insufficiently reported)**
	Reverse mutation	*S.* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	0.32–5,000 μg/plate[(a),(b)]	Negative	Ballantyne (2011)	Evidence of toxicity was observed at 1,000 and/or 5,000 μg/plate in the absence and presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved
		S. Typhimurium TA98, TA100, TA102, TA1535 and TA1537	10.24–1,000 μg/plate[(b),(d)] or [(c),(e)]	Negative		Evidence of toxicity was observed in all strains at 1,000 μg/plate and in strains TA100 and TA102 as low as 160 μg/plate in the absence and presence of S9-mix. Study design complied with current recommendations. Acceptable top concentration was achieved
	Micronucleus assay	Human peripheral blood lymphocytes	30–60 μg/mL[(d),(f)] 80–120 μg/mL[(e),(f)] 5–17.5 μg/mL[(d),g)]	Negative	Stone (2011)	The top concentrations induced 50–60% toxicity. The MNBN cell frequencies in all treated cultures fell within the normal range. Complies with draft OECD Guideline 487

OECD: Organisation for Economic Cooperation and Development; MNBN: micronucleated binucleate cells.
(a): With and without S-9 metabolic activation.
(b): Plate incorporation method.
(c): Pre-incubation method.
(d): Without S-9 metabolic activation.
(e): With S-9 metabolic activation.
(f): 3-h incubation with 21-h recovery period.
(g): 24-h incubation with no recovery period.

Table C.4: Summary of *in vitro* genotoxicity data evaluated in FGE.208Rev2 (EFSA CEF Panel, 2017)

Chemical name [FL-no]	Test system	Test object	Concentration	Results	Reference	Comments
***p*-Mentha-1,8-dien-7-ol [FL-no: 02.060]**	Bacterial reverse mutation assay	*S.* Typhimurium TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvr*A	10, 33.3, 100, 333, 1,000, 3,333 μg/plate[(a),(b)]	Negative[(a),(b)]	Wagner (2016)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 471. Toxicity at concentrations ≥ 3,333 μg/plate
			1, 3.33, 10, 33.3, 100, 333, 1,000, 3,333 μg/plate[(a),(f)]	Negative[(a),(f)]		Toxicity at concentrations ≥ 100 or 333 μg/plate
	Micronucleus assay	Human peripheral blood lymphocytes	25, 50 and 100 μg/mL[(c)]	Negative	Roy (2016)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 487
			100, 250 and 325 μg/mL[(g)]			
			100, 25, 275 μg/mL[(h)]			
Myrtenol [02.091]	Bacterial reverse mutation assay	*S.* Typhimurium TA98, TA100, TA1535, TA1537	5, 16, 50, 160, 500, 1,600, 5,000 μg/plate[(a),(b)]	Negative[(a),(b)]	Bhalli and Phil (2015a)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 471
		E. coli WP2*uvr*A	5, 16, 50, 160, 500, 1,600, 5,000 μg/plate[(a),(b)]	Negative[(a),(b)]		
		S. Typhimurium TA98, TA100, TA1535, TA1537	16, 50, 160, 500, 1,600, 5,000 μg/plate	Negative[(a),(b)]		
		E. coli WP2uvrA	16, 50, 160, 500, 1,600, 5,000 μg/plate	Negative[(a),(b)]		
	BluScreen™ HC	Human lymphoblastoid TK6 cells	9.77, 19.53, 39.06, 78.13, 156.25, 312.50, 625, 1,250 μM	Negative[(a)]	Birrell (2013a)	The reliability was not evaluated since this assay does not belong to the assays recommended by the Scientific Committee for regulatory purposes (EFSA Scientific Committee, 2011)
	Micronucleus assay	Human peripheral blood lymphocytes	30.6, 47.2 and 52.5 μg/mL[(c)] 368, 387, 451 and 475 μg/mL[(d)] 407, 451 and 475 μg/mL[(e)]	Negative[(c),(d),(e)]	Bhalli and Phil (2015b)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 487

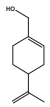
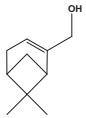
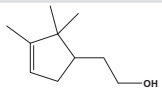
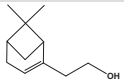
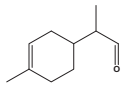
Chemical name [FL-no]	Test system	Test object	Concentration	Results	Reference	Comments
Myrtenal [05.106]	Bacterial reverse mutation assay	*S.* Typhimurium TA98, TA100, TA102, TA1535, TA1537	5, 16, 50, 160, 500, 1,600 and 5,000 µg/plate[(a),(b)]	Negative[(a),(b)]	Mc Garry (2016c)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 471
			80, 160, 300, 625, 1,250, 2,500 and 5,000 µg/plate	Negative[(a),(b),(f)]		
	Micronucleus assay	Human peripheral blood lymphocytes	15, 25 and 34 µg/mL[(c)] 50, 130 and 180 µg/mL[(d)] 25, 200 and 350 µg/mL[(e)]	Equivocal[(c),(d),(e)]	Mc Garry (2016d)	Reliable with restriction. GLP study in compliance with OECD Test Guideline 487
	Micronucleus assay	Human peripheral blood lymphocytes		Equivocal[(c),(d),(e),(i)]	Lloyd (2017)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 487
***p*-Mentha-1,8-dien-7-yl acetate [FL-no: 09.278]**	Bacterial reverse mutation assay	*S.* Typhimurium TA98, TA100, TA102, TA1535, TA1537	5, 16, 50, 160, 500, 1600, and 5,000 µg/plate[(a),(b)]	Negative[(a),(b)]	Lloyd (2016a)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 471
			1.6, 5, 16, 50, 160, 500 and 1,600 µg/plate[(a),(b),(f)]	Negative[(a),(b),(f)]		
	Micronucleus assay	Human peripheral blood lymphocytes	30, 60 and 80 µg/mL[(c)] 100, 120 and 130 µg/mL[(d)] 150, 240 and 280 µg/mL[(e)]	Negative[(c),(d),(e)]	Lloyd (2016b)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 487

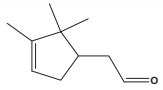
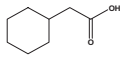
Chemical name [FL-no]	Test system	Test object	Concentration	Results	Reference	Comments
Myrtenyl acetate [09.302]	Bacterial reverse mutation assay	*S.* Typhimurium TA98, TA100, TA102, TA1535, TA1537	5, 16, 50, 160, 500, 1600, and 5,000 μg/plate[(a),(b)]	Negative[(a),(b)]	Mc Garry (2016a)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 471
			3.28, 8.2, 20.5, 51.2, 128, 320, 800 and 2,000 μg/plate	Negative[(a),(b),(f)]		
	BluScreen™ HC	Human lymphoblastoid TK6 cells	4.88, 9.77, 19.53, 39.06, 78.13, 156.25, 312.50, 625 μM	Negative[(a)]	Birrell (2013b)	The reliability was not evaluated since this assay does not belong to the assays recommended by the Scientific Committee for regulatory purposes (EFSA Scientific Committee, 2011)
	Micronucleus assay	Human peripheral blood lymphocytes	10, 20 and 40 μg/mL[(c)] 20, 60, 80 and 90 μg/mL[(d)] 150, 250 and 325 μg/mL[(e)]	Negative[(c),(d),(e)]	Mc Garry (2016b)	Reliable without restriction. GLP study in compliance with OECD Test Guideline 487

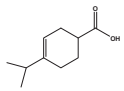
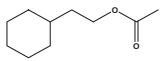
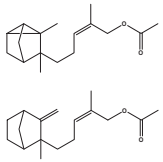
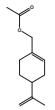
GLP: Good laboratory practice; OECD: Organisation for Economic Cooperation and Development.
(a): Assay performed with and without metabolic activation.
(b): Plate incorporation method.
(c): 24 h treatment without metabolic activation, with no recovery.
(d): 3 h treatment without metabolic activation, with 21 h recovery.
(e): 3 h treatment with metabolic activation, with 21 h recovery.
(f): Pre-incubation method applied in the presence of metabolic activation.
(g): 4 h treatment without metabolic activation, with 20 h recovery.
(h): 4 h treatment with metabolic activation, with 20 h recovery.
(i): 24 h treatment, without metabolic activation, with 24 h recovery.

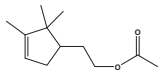
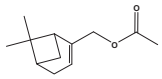
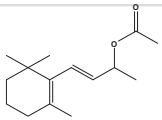
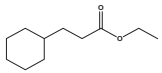
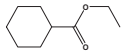
Appendix D – Summary of the safety evaluation

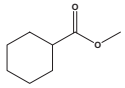
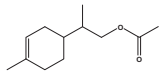
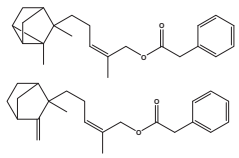
Table D.1: Summary of safety evaluation for the JECFA-evaluated substances in FGE.73.

FL-no JECFA-no	EU register name	Structural formula	EU MSDI [(a)] US MSDI (μg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	EFSA conclusion on the named compound (procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.060 974	*p*-Mentha-1,8-dien-7-ol		0.34 1.0	Class I A3: Intake below TTC	d	Evaluated in FGE.208Rev2, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
02.091 981	Myrtenol		0.1 0.03	Class I A3: Intake below TTC	d	Evaluated in FGE.208Rev2, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
02.114 970	2-(2,2,3-Trimethylcyclopent-3-enyl)ethan-1-ol		0.012 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
02.141 986	2-(6,6-Dimethylbicyclo [3.1.1]hept-2-en-2-yl)ethan-1-ol		33 0.01	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
05.098 971	*p*-Menth-1-en-9-al		0.12 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach

FL-no JECFA-no	EU register name	Structural formula	EU MSDI [a] US MSDI (μg/*capita* per day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	EFSA conclusion on the named compound (procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
05.112 978	2,6,6-Trimethylcyclohex-1-en-1-acetaldehyde		0.24 2	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	According to JECFA: Min. assay value is 92%'. Secondary components β-cyclocitral (2–3%), β-ionone (0.5–1%), methyl β-homocyclogeranate (2–4%), ethyl β-homocyclogeranate (0.6–1%) (EFFA, 2010a) No safety concern at the estimated level of intake based on the MSDI approach
05.119 967	2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde		5 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	CASrn in Register refers to (*R*)-isomer. Register name to be changed to (1*R*) 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde. No safety concern at the estimated level of intake based on the MSDI approach
05.123 968	5-Isopropenyl-2-methylcyclopentanecarboxaldehyde		0.012 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	CASrn in Register refers to (1*R,*2*R,*5S)-isomer. Register name to be changed to (1*R,*2*R,*5S) 5-isopropenyl-2-methylcyclopentanecarboxaldehyde No safety concern at the estimated level of intake based on the MSDI approach
08.034 965	Cyclohexylacetic acid		0.12 0.4	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach

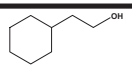
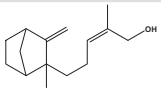
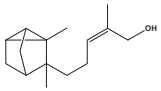
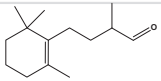
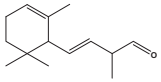
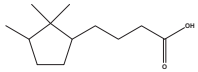
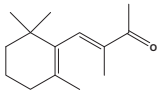
FL-no JECFA-no	EU register name	Structural formula	EU MSDI [(a)] US MSDI (μg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
08.060 961	Cyclohexanecarboxylic acid		0.061 4	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
08.067 976	1,2,5,6-Tetrahydrocuminic acid		0.012 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
09.028 964	2-Cyclohexylethyl acetate		0.97 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
09.034 985	Santalyl acetate		0.1 0.01	Class I A3: Intake below TTC	d	Evaluated in FGE.207, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach	CASrn in Register refers to incompletely defined substance. According to JECFA: Min. assay value is '95%' and secondary components '60–65% alpha, 30–35% beta form' No safety concern at the estimated level of intake based on the MSDI approach
09.278 975	*p*-Mentha-1,8-dien-7-yl acetate		1.4 0.07	Class I A3: Intake below TTC	d	Evaluated in FGE.208Rev2, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach	Stereoisomeric composition to be specified

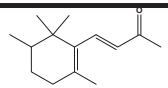
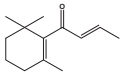
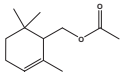
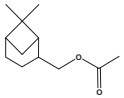
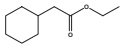
FL-no JECFA-no	EU register name	Structural formula	EU MSDI [a] US MSDI (μg/*capita* per day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	EFSA conclusion on the named compound (procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.289 969	alpha-Campholene acetate		0.061 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	Register name to be changed to (-)-campholenyl acetate or (S)-campholenyl acetate. No safety concern at the estimated level of intake based on the MSDI approach
09.302 982	Myrtenyl acetate		0.91 0.04	Class I A3: Intake below TTC	d	Evaluated in FGE.208Rev2, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
09.305 1409	beta-Ionyl acetate		3.3 9	Class I A3: Intake below TTC	d	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out	Acc. to JECFA: Min. assay value is '92%' and secondary components '2–3% acetic acid; 1–2% beta-ionol'. Racemate, the double bond is mainly E-isomer: E/Z ratio about 50-70%/30-50%. (EFFA, 2014a). No safety concern at the estimated level of intake based on the MSDI approach
09.488 966	Ethyl cyclohexanepropionate		0.12 0.1	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
09.534 963	Ethyl cyclohexanecarboxylate		0.24 0.1	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach

FL-no JECFA-no	EU register name	Structural formula	EU MSDI [(a)] US MSDI (μg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.536 962	Methyl cyclohexanecarboxylate		0.073 0.01	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
09.615 972	*p*-Menth-1-en-9-yl acetate		0.85 ND	Class I A3: Intake below TTC	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
09.712 1022	Santalyl phenylacetate		0.029 1	Class I A3: Intake below TTC	d	Evaluated in FGE.207, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach	60–65% alpha-, 30–35% beta-form. 80–85% *Z* vs 15–20% *E* (for the alpha) and 75–80% *Z* vs 20–25% – (for the beta) (EFFA, 2013). No safety concern at the estimated level of intake based on the MSDI approach
05.104 977	2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde		3.5 0.06	Class I B3: Intake below TTC, B4: Adequate NOAEL exists	d	Evaluated in FGE.209, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach

ND: Not determined.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = μg/*capita* per day.
(b): TTCs of concern: Class I = 1,800 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.

Table D.2: Summary of safety evaluation by the EFSA (FGE.12Rev5) (EFSA CEF Panel, 2014b)

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) or (e)]	Outcome on the material of commerce [(f),(g) or (h)]	Evaluation remarks
02.134	2-Cyclohexylethan-1-ol		0.011	Class I A3: Intake below TTC	d	f	
02.186	Myrtanol		0.37	Class I A3: Intake below TTC	d	f	
02.216	12-beta-Santalen-14-ol		0.085	Class I A3: Intake below TTC	d	f	
02.217	12-alpha-Santalen-14-ol		0.11	Class I A3: Intake below TTC	d	f	
05.157	Isocyclocitral		0.011	Class I A3: Intake below TTC	d	f	
05.182	2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde		0.061	Class I A3: Intake below TTC	d	f	
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal		0.012	Class I A3: Intake below TTC	d	f	
05.198	alpha-Methyl ional		0.011	Class I A3: Intake below TTC	d	f	
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid		43	Class I A3: Intake below TTC	d	f	
07.041	beta-Isomethylionone		0.011	Class I A3: Intake below TTC	d	f	

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) or (e)]	Outcome on the material of commerce [(f),(g) or (h)]	Evaluation remarks
07.200	4-(2,5,6,6-Tetramethyl-1-cyclohexenyl)but-3-en-2-one		0.012	Class I A3: Intake below TTC	d	f	
07.224	tr-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)but-2-en-1-one		100	Class I A3: Intake below TTC	d	f	
09.342	Cyclogeranyl acetate		0.24	Class I A3: Intake below TTC	d	f	
09.670	Myrtanyl acetate		0.58	Class I A3: Intake below TTC	d	f	
09.829	Ethyl cyclohexyl acetate		0.61	Class I A3: Intake below TTC	d	f	

(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = μg/*capita* per day.
(b): Toxicological thresholds of concern (TTC): Class I = 1,800 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.