EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 87, Revision 2 (FGE.87Rev2): Consideration of bicyclic secondary alcohols, ketones and related esters evaluated by JECFA (63rd meeting) structurally related to bicyclic secondary alcohols, ketones and related esters evaluated by EFSA in FGE.47Rev1 (2008)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 87, Revision 2 (FGE.87Rev2): Consideration of bicyclic secondary alcohols, ketones and related esters evaluated by JECFA (63rd meeting) structurally related to bicyclic secondary alcohols, ketones and related esters evaluated by EFSA in FGE.47Rev1 (2008)[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 19 bicyclic secondary alcohols, ketones and related esters evaluated by the JECFA at the 63rd meeting in 2004. This revision of FGE.87 is made due to inclusion of two additional substances Nookatone [FL-no: 07.089] and 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136] cleared for genotoxicity concern in FGE.213 Rev1. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for all substances considered in this FGE and for 18 substances the Panel agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. For one substance [FL-no: 07.136], a production volume for Europe is not available, which precludes the finalisation of the evaluation by EFSA of this substance. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered for the substances evaluated through the Procedure and for all 19 substances, the information is adequate.

[1] On request from the European Commission, Question No EFSA-Q-2014-00348 and EFSA-Q-2014-00349, adopted on 25 September 2014.

[2] Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Wim Mennes, Maria Rosaria Milana, André Penninks, Vittorio Silano, Andrew Smith, Maria de Fátima Tavares Poças, Christina Tlustos, Fidel Toldrá, Detlef Wölfle and Holger Zorn. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft, Pia Lund and Karin Nørby and EFSA staff: Maria Carfí, Annamaria Rossi and Kim Rygaard Nielsen for the support provided to this scientific opinion.

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KEY WORDS

flavouring, bicyclic, secondary alcohols, ketones, esters, FGE.47, FGE.87

SUMMARY

Following a request from the European Commission the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the CEF Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

This consideration deals with 19 bicyclic secondary alcohols, ketones and related esters, which are in the Register and which were evaluated by the JECFA at its 63rd meeting.

The revision is made due to consideration of two additional substances nootkatone and 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.089 and 07.136] compared to the previous version. These two substances are α,β-unsaturated alicyclic ketones which have been considered with respect to genotoxicity in FGE.213Rev1, and the Panel concluded that the data available ruled out the concern for genotoxicity and thus concluded that the two substances can be evaluated through the Procedure in this FGE.87Rev2.

The Panel concluded that all 19 substances are structurally related to the group of bicyclic secondary alcohols, ketones and related evaluated by EFSA in the Flavouring Group Evaluation 47 (FGE.47Rev1).

The Panel agrees with the application of the Procedure as performed by the JECFA for the 19 bicyclic secondary alcohols, ketones and related esters. It was concluded at step A3 of the Procedure that 18 substances do not pose a safety concern when used as flavouring substances at estimated levels of intake, based on the MSDI approach. For one substance [FL-no: 07.136], the evaluation through the Procedure could not be finalised because of absence of an EU production volume.

For all 19 substances evaluated through the Procedure use levels are needed to calculate the modified Theoretical Added Maximum Daily Intake (mTAMDIs)in order to identify those flavouring substances that need more refined exposure assessments and to finalise the evaluation.

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 are available for all the materials of commerce, however, a production volume for EU for substance [FL-no: 07.136] is not available, which precludes the finalisation of the evaluation by EFSA of this substance.

For the remaining 18 JECFA evaluated bicyclic secondary alcohols, ketones and related esters [FL-no: 02.016, 02.038, 02.059, 02.100, 02.101, 07.089, 07.153, 07.159, 09.017, 09.082, 09.131, 09.153, 09.176, 09.218, 09.269, 09.319, 09.456 and 09.457] the Panel agrees with the JECFA conclusion: “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] 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[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA concluded that a genotoxic potential of the α,β-unsaturated precursor, beta-ionyl acetate [FL-no: 09.305] in FGE.213 could not be ruled out.

Information on four representative materials has now been submitted by the European Flavour Association. These are beta-ionone [FL-no: 07.008], maltol [FL-no: 07.014], nootkatone [FL-no: 07.089] and 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109].

This information is intended to cover also the re-evaluation of the following eight substances from FGE.19 subgroup 2.7:

- 4-(2,2,6-Trimethyl-1-cyclohexenyl)but-3-en-2-ol [FL-no: 02.106]
- Methyl-beta-ionone [FL-no: 07.010]
- Beta-Isomethylionone [FL-no: 07.041]
- P-Mentha-1,4(8)-dien-3-one [FL-no: 07.127]
- 4,4a,5,6-Tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136]
- 4-(2,5,6,6-Tetramethyl-1-cyclohexenyl)but-3-en-2-one [FL-no: 07.200]
- beta-Ionyl acetate [FL-no: 09.305]
- Maltyl isobutyrate [FL-no: 09.525]

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substance.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests European Food Safety Authority to carry out a safety assessment on the following 12 flavouring substances: 4-(2,2,6-trimethyl-1-cyclohexenyl)but3-en-2-ol [FL-no: 02.106], beta-ionone [FL-no: 07.008], methyl-beta-ionone [FL-no: 07.010], maltol [FL-no: 07.014], beta-isomethylionone [FL-no: 07.041], nootkatone [FL-no: 07.089], 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109], p-mentha-1,4(8)-dien-3-one [FL-no: 07.127], 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136], 4-(2,5,6,6-tetramethyl-1-cyclohexenyl)but-3-en-2-one [FL-no: 07.200], beta-ionyl acetate [FL-no: 09.305], maltyl isobutyrate [FL-no: 09.525] in accordance with Commission Regulation (EC) No 1565/2000.

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

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

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

INTERPRETATION OF THE TERMS OF REFERENCE

Nootkatone [FL-no: 07.089] and 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136] were first allocated to FGE.213Rev1 for evaluation with respect to genotoxicity. Based on the new genotoxicity data submitted, the Panel concluded that [FL-no: 07.089] and [FL-no: 07.136] do not give rise to concern with respect to genotoxicity and can accordingly now be evaluated through the Procedure in FGE.87Rev2.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000, 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; JECFA, 1996; JECFA, 1997; JECFA, 1999), hereafter named the "JECFA Procedure". The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the 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 evaluated 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, the 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 the 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 the JECFA only on the basis of these figures. For Register substances for which this is the case, the Panel will need 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 the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the 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, 2006b).

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 the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram (µg)/person/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 per 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 threshold of 1.5 µg per person per day.

Genotoxicity

As reflected in the Opinion of SCF (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 FGE.

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

At its 63rd meeting the JECFA evaluated a group of 32 flavouring substances consisting of monocyclic and bicyclic secondary alcohols, ketones and related esters. Three substances were not in the Register, and six are α,β-unsaturated ketones or precursors for such and these will be or have been considered together with other α,β unsaturated substances aldehydes and ketones (EFSA, 2008b) in FGE.211, FGE.212 and FGE.213 and revisions thereof. One is an ether [FL-no: 16.088] considered in a revision of FGE.59 (FGE.59Rev1). Six are monocyclic secondary alcohols, ketones and related esters considered in FGE.56. Finally, the JECFA evaluated substance (1R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (camphor [FL-no: 07.215]), which the Panel has evaluated in a separate Opinion (EFSA, 2008c). The remaining 15 flavouring substances were considered by EFSA in FGE.87 (EFSA, 2008a).

The first revision of FGE.87, FGE.87Rev1 included the consideration of additional two substances [FL-no: 02.100 and 02.101]. These two substances are precursors for α,β-unsaturated ketones and were originally allocated to FGE.211 and FGE.212, respectively. Since the publication of FGE.87, the EU production volumes were provided for two substances, [FL-no: 09.153 and 09.319] for which the evaluation could not be finalised due to lack of these data. Based on these newly submitted EU production volumes the substances have already been evaluated in FGE.96 (EFSA, 2010), but for the sake of completion, the information has also been included here as well. Finally, new information on the stereoisomeric composition has been provided for 13 substances [FL-no: 02.016, 02.038, 02.059, 07.159, 09.017, 09.082, 09.131, 09.153, 09.176, 09.218, 09.319 and 09.456 and 09.457] since the publication of FGE.87 (EFFA, 2010a, 2011).

FGE	Opinion adopted	Link	No. of substances
FGE.87	22 May 2008	http://www.efsa.europa.eu/en/efsajournal/pub/746.htm	15
FGE.87Rev1	1 February 2012	http://www.efsa.europa.eu/en/efsajournal/doc/2564.pdf	17
FGE.87Rev2	2014		19

The present revision of FGE.87 (FGE.87Rev2) concerns the consideration of two JECFA-evaluated substances nootkatone and 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.089 and 07.136].

They were both evaluated by the JECFA at its 63rd meeting together with other monocyclic and bicyclic secondary alcohols, ketones and related esters. Both are α,β-unsaturated alicyclic ketones and were originally allocated to and evaluated in FGE.213Rev1 (EFSA CEF Panel, 2014b) in which they were considered not to be of concern with respect to genotoxicity. The Panel concluded that the substances could be included in the present FGE.87Rev2.

For two substances [FL-no: 02.100] and [FL-no: 02.101] the information on stereoisomeric composition has been received and included in this FGE.

2. Presentation of the Substances in the JECFA Flavouring Group

2.1. Description

2.1.1. JECFA Status

The JECFA has at the 63rd meeting evaluated a group of 32 flavouring substances consisting of monocyclic and bicyclic secondary alcohols, ketones and related esters (JECFA, 2006a).

2.1.2. EFSA Considerations

Three of the 32 JECFA evaluated substances are not included in the Register, alpha-isomethylionyl acetate (JECFA-no: 1410), *d,l*-menthol-(±)-propylene glycol carbonate (JECFA-no: 1413) and *l*-monomenthyl glutarate (JECFA-no: 1414).

Six of the 32 JECFA evaluated substances are α,β-unsaturated [FL-no: 02.100, 02.101, 07.089, 07.136, 07.140 and 09.305] and will be or have been evaluated together with other α,β-unsaturated aldehydes and ketones (EFSA, 2008b). However, four of these α,β-unsaturated substances [FL-no: 02.100, 02.101, 07.089 and 07.136] have been considered with respect to genotoxicity in FGE.211 (EFSA CEF Panel, 2011), FGE.212Rev2 (EFSA CEF Panel, 2014a) and FGE.213Rev1 (EFSA CEF Panel, 2014b) where the Panel concluded that the data available did rule out the concern for genotoxicity and thus concluded that these four substances can be evaluated through the Procedure in FGE.87.

One of the JECFA evaluated substances is an ether [FL-no: 16.088] which is considered together with other ethers in a revision of FGE.59 (FGE.59Rev1). Six of the JECFA evaluated substances are monocyclic secondary alcohols, ketones and related esters and are considered in FGE.56. Finally, the JECFA evaluated substance, (1R)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one (camphor [FL-no: 07.215]), has been evaluated by the Panel in a separate Opinion (EFSA, 2008c).

This consideration will therefore deal with 19 bicyclic secondary alcohols, ketones and related esters consisting of the 15 substances originally considered in FGE.87 and the four α,β-unsaturated substances, which have been cleared for concern for genotoxicity. The Panel concluded that all substances in the JECFA flavouring group of bicyclic secondary alcohols, ketones and related esters are structurally related to the group of four bicyclic secondary alcohols, ketones and related esters evaluated by EFSA in FGE.47Rev1.

2.2. Isomers

2.2.1. Status

All 19 substances have one or more chiral centres (see Table 1).

2.2.2. EFSA Considerations

The available specifications are considered adequate for all 19 substances.

For the two stereoisomeric substances [FL-no: 07.153 and 09.269] with one chiral centre, the CAS register number (CASrn) is considered to cover the stereoisomeric composition.

2.3. Specifications

2.3.1. Status

JECFA specifications are available for all substances (JECFA, 2005a).

2.3.2. EFSA Considerations

The information provided is adequate for all substances.

3. Intake Estimation

3.1. Status

For 18 substances evaluated through the JECFA Procedure production figures are available for the EU. No EU production volume is available for the flavouring substance 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136] (see Table 1).

3.2. EFSA Considerations

For the flavouring substance 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136] no EU production volume is available to calculate an MSDI exposure estimate. For all substances, use levels are needed to calculate the mTAMDIs.

Table 1: Estimated intakes based on the MSDI- and the mTAMDI approach – FGE.87Rev2

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.016	Borneol	130	23		Class I	1800
02.038	Fenchyl alcohol	55	17		Class I	1800
02.059	Isoborneol	21	0.07		Class I	1800
02.100	Pinocarveol	0.012	0.01		Class I	1800
02.101	Pin-2-en-4-ol	0.012	0.2		Class I	1800
09.017	Bornyl acetate	18	3		Class I	1800
09.082	Bornyl formate	1.2	0.09		Class I	1800
09.131	Isobornyl propionate	2.6	0.007		Class I	1800
09.153	Bornyl valerate	3.7	5		Class I	1800
09.176	Isobornyl formate	0.61	0.4		Class I	1800
09.218	Isobornyl acetate	890	236		Class I	1800
09.269	Fenchyl acetate	2.9	0.07		Class I	1800
09.319	Bornyl butyrate	6.1	9		Class I	1800
09.456	Bornyl isovalerate	0.12	0.5		Class I	1800
09.457	Isobornyl isovalerate	0.012	0.08		Class I	1800
07.089	Nootkatone	130	20		Class II	540
07.136	4,4a,5,6-Tetrahydro-7-methylnapthalen-2(3H)-one		0.04		Class II	540
07.153	1,10-Dihydronootkatone	0.24	0.9		Class II	540
07.159	*d*-Fenchone	6.3	5		Class II	540

SUMMARY OF SPECIFICATION DATA

Table 2: Specification Summary of the Substances in the JECFA Flavouring Group of Bicyclic Secondary Alcohols, Ketones and Related Esters (JECFA, 2005a)

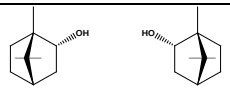
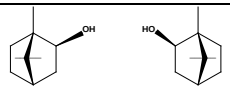
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec. gravity [e]	EFSA comments
02.016 1385	Borneol		2157 64 507-70-0	Solid $C_{10}H_{18}O$ 154.25	Very slightly soluble Soluble	n.a. 202 IR 97 %	n.a. n.a.	Racemate (±) = *DL*-Borneol (EFFA, 2010a). CASrn refers to (1*R*,2*S*,4*R*)-rel. Register name to be changed to *DL*-Borneol (EFFA, 2011). According to JECFA "Min. Assay value may incl. Isoborneol, other isomers of borneol, trace amounts of fenchyl alcohol & other C10H18O compounds".
02.038 1397	Fenchyl alcohol		2480 87 1632-73-1	Solid $C_{10}H_{18}O$ 154.25	Very slightly soluble Soluble	n.a. 35-40 IR 97 %	n.a. n.a.	Racemate (EFFA, 2010a). According to JECFA "Min. Assay value is (97 %) of C10H18O which may include small amounts of borneol and isoborneol".
02.059 1386	Isoborneol		2158 2020 124-76-5	Solid $C_{10}H_{18}O$ 154.25	Very slightly soluble Soluble	n.a. 212-214 IR 92 %	n.a. n.a.	Racemate (±) = *DL*-isoborneol (EFFA, 2011). CASrn in Register refers to (1*R*,2*R*,4*R*)-rel.

Table 2: Specification Summary of the Substances in the JECFA Flavouring Group of Bicyclic Secondary Alcohols, Ketones and Related Esters (JECFA, 2005a)

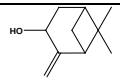
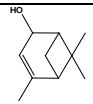
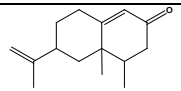
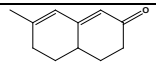
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec. gravity [e]	EFSA comments
								Register name to be changed to *DL*-Isoborneol (EFFA, 2011). According to JECFA: Min. assay value is ”92 %” and secondary components ”3-5 % borneol”.
02.100 1403	Pinocarveol		3587 10303 5947-36-4	Liquid $C_{10}H_{16}O$ 152.24	Insoluble Soluble	210 NMR 95 %	1.445-1.451 0.977-0.983	Racemate (EFFA, 2014).
02.101 1404	Pin-2-en-4-ol		3594 10304 473-67-6	Solid $C_{10}H_{16}O$ 152.24	Very slightly soluble Soluble	n.a. 63-67 NMR 95 %	n.a. n.a.	Racemate (EFFA, 2014).
07.089 1398	Nootkatone		3166 11164 4674-50-4	Liquid $C_{15}H_{22}O$ 218.35	Slightly soluble Soluble	73-103 (1 hPa) NMR 93 %	1.510-1.523 1.003-1.032	(+)-Nootkatone which refers to the (4*R*,4a*S*,6*R*)-isomer (EFFA, 2014). According to JECFA: Min. assay value is ”93 %” and secondary components ”2-3 % dihydronootkatone”.
07.136 1405	4,4a,5,6-Tetrahydro-7-methylnapthalen-2(3H)-one		3715 34545-88-5	Solid $C_{11}H_{14}O$ 162.23	Insoluble Soluble	n.a. 36-37 IR 99 %	n.a. n.a.	Racemate (EFFA, 2014). Name to be changed to: 4,4a,5,6-Tetrahydro-7-methylnaphthalen-2(3H)-one

Table 2: Specification Summary of the Substances in the JECFA Flavouring Group of Bicyclic Secondary Alcohols, Ketones and Related Esters (JECFA, 2005a)

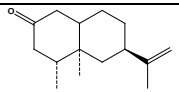
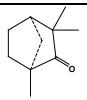
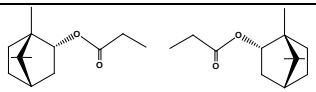
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec. gravity [e]	EFSA comments
07.153 1407	1,10-Dihydronootkatone		3776 20489-53-6	Liquid $C_{15}H_{24}O$ 220.36	Very slightly soluble Soluble	100-104(0.09hPa NMR 90 %	1.502-1.508 0.975-0.988	CASrn in Register refers to (4*R*,4a*S*,6*R*,8a*S*)-stereoisomer. According to JECFA ”Min. assay value is (90 %) and secondary components (5-6% nootkatone)”.
07.159 1396	*d*-Fenchone		2479 551 4695-62-9	Liquid $C_{10}H_{16}O$ 152.24	Insoluble Soluble	192 IR 97 %	1.460-1.467 0.940-0.948	*D*-(+)-Fenchone (EFFA, 2010a). CASrn in Register refers to (1*S*,4*R*)-isomer. According to JECFA ”Min. Assay value is ”97 % of C10H16O” which may include small amounts of *d*-camphor”.
09.017 1387	Bornyl acetate		2159 207 76-49-3	Liquid $C_{12}H_{20}O_2$ 196.29	Slightly soluble Soluble	226 25 IR 98 %	1.462-1.466 0.981-0.985	Racemate (±) = *DL*-Bornyl acetate (EFFA, 2010a). CASrn in Register refers to (1*R*,2*S*,4*R*)-rel. Register name to be changed to *DL*-Bornyl acetate (EFFA, 2011).

Table 2: Specification Summary of the Substances in the JECFA Flavouring Group of Bicyclic Secondary Alcohols, Ketones and Related Esters (JECFA, 2005a)

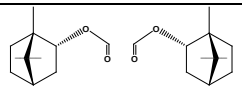
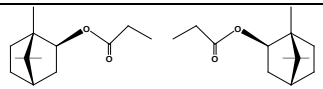
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec. gravity [e]	EFSA comments
								According to JECFA "Min. Assay value is 98 % and may include isobornyl acetate and other bornyl acetate isomers".
09.082 1389	Bornyl formate		2161 349 7492-41-3	Liquid $C_{11}H_{18}O_2$ 182.26	Slightly soluble Soluble	106-108 (27hPa) NMR 95 %	1.466-1.472 1.007-1.013 (20°)	Racemate (±) = *DL*-Bornyl formate (EFFA, 2011). CASrn in Register refers to (1*R*,2*S*,4*R*)-rel. Register name to be changed to DL-Bornyl formate (EFFA, 2011).
09.131 1391	Isobornyl propionate		2163 412 2756-56-1	Liquid $C_{13}H_{22}O_2$ 210.32	Soluble Soluble	245 NMR 97 %	1.461-1.465 0.968-0.971	Racemate (±) = *DL*-Isobornyl propionate (EFFA, 2010a). CASrn in Register refers to (1*R*,2*R*,4*R*)-rel.Register name to be changed to *DL*-Isobornyl propionate (EFFA, 2011). According to JECFA "Min. Assay value may include small amounts of bornyl propionate".

Table 2: Specification Summary of the Substances in the JECFA Flavouring Group of Bicyclic Secondary Alcohols, Ketones and Related Esters (JECFA, 2005a)

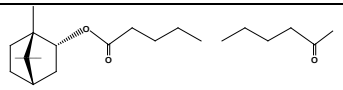
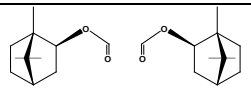
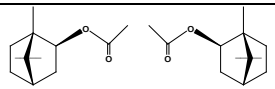
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec. gravity [e]	EFSA comments
09.153 1392	Bornyl valerate		2164 471 7549-41-9	Liquid $C_{15}H_{26}O_2$ 238.37	Insoluble Soluble	136-137 (16hPa) NMR 96 %	1.459-1.465 0.957-0.963	Racemate (±) = *DL*-Bornyl valerate (EFFA, 2010a). CASrn in Register refers to (1R,2S,4R)-rel. Register name to be changed to *DL*-Bornyl valerate (EFFA, 2011).
09.176 1390	Isobornyl formate		2162 565 1200-67-5	Liquid $C_{11}H_{18}O_2$ 182.26	Slightly soluble Soluble	94-95 (20 hPa) NMR 96 %	1.469-1.473 1.011-1.017	Racemate (±) = *DL*-Isobornyl formate (EFFA, 2010a). CASrn in Register refers to (1*R*,2*R*,4*R*)-rel. Register name to be changed to *DL*-Isobornyl formate (EFFA, 2011). According to JECFA: Min. Assay value "may include small amounts of bornyl formate".
09.218 1388	Isobornyl acetate		2160 2066 125-12-2	Liquid $C_{12}H_{20}O_2$ 196.29	Insoluble Soluble	227 IR 97 %	1.462-1.465 0.979-0.984	Racemate (±) = *DL*-Isobornyl acetate (EFFA, 2010a). CASrn in Register refers to (1*R*,2*R*,4*R*)-rel. Register name to

Table 2: Specification Summary of the Substances in the JECFA Flavouring Group of Bicyclic Secondary Alcohols, Ketones and Related Esters (JECFA, 2005a)

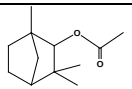
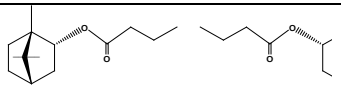
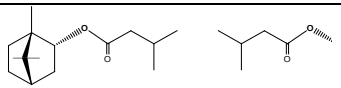
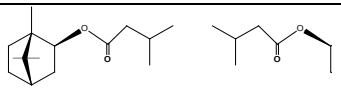
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec. gravity [e]	EFSA comments
								be changed to *DL*-Isobornyl acetate (EFFA, 2011). According to JECFA "Min. Assay value may include small amounts of bornyl acetate".
09.269 1399	Fenchyl acetate		3390 11769 13851-11-1	Liquid $C_{12}H_{20}O_2$ 196.29	Slightly soluble Soluble	220 NMR 98 %	1.456-1.462 0.973-0.979	Racemate. (CASrn in Register refers to the racemate).
09.319 1412	Bornyl butyrate		3907 13109-70-1	Liquid $C_{14}H_{24}O_2$ 224.34	Slightly soluble Soluble	247 MS 97 %	1.462-1.469 0.981-0.991	Racemate (±) = *DL*-Bornyl butyrate. CASrn in Register refers to (1*R*,2*S*,4*R*)-rel. Register name to be changed to *DL*-Bornyl butyrate (EFFA, 2011).
09.456 1393	Bornyl isovalerate		2165 451 76-50-6	Liquid $C_{15}H_{26}O_2$ 238.37	Insoluble Soluble	260 NMR 97 %	1.458-1.461 0.944-0.947	Racemate (±) = *DL*-Bornyl isovalerate. CASrn in Register refers to (1*R*,2*S*,4*R*)-rel. Register name to be changed to *DL*-Bornyl isovalerate (EFFA, 2011).

Table 2: Specification Summary of the Substances in the JECFA Flavouring Group of Bicyclic Secondary Alcohols, Ketones and Related Esters (JECFA, 2005a)

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]	EFSA comments
09.457 1394	Isobornyl isovalerate		2166 452 7779-73-9	Liquid $C_{15}H_{26}O_2$ 238.37	Insoluble Soluble	266-269 NMR 96 %	1.463-1.469 0.900-0.906	Racemate (±) = *DL*-Isobornyl isovalerate. CASrn in Register refers to (1*R*,2*R*,4*R*)-rel. Register name to be changed to *DL*-Isobornyl isovalerate (EFFA, 2011).

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.
n.a. not applicable.

4. Genotoxicity Data

4.1. Genotoxicity Studies - Text Taken[7] from the JECFA (JECFA, 2006a)

Tests for genotoxicity *in vitro* and *in vivo* using standardized protocols have been used to study two representative members [FL-no: 02.016 and 09.131] of the bicyclic secondary alcohols, ketones and related esters group used as flavouring agents.

In vitro

Two members of this group (borneol, [FL-no: 02.016] and isobornyl propionate, [FL-no: 09.131]) consistently gave negative results in the Ames assay when incubated at a concentration of up to 5000 µg/plate with a variety of *Salmonella typhimurium* strains including TA97, TA98, TA100, TA1535, TA1537 and TA1538 with or without metabolic activation (Simmon et al., 1977; Wild et al., 1983; Azizan and Blevins, 1995).

Borneol [FL-no: 02.016] showed no mutagenic activity when tested in *Escherichia coli* WP2 *uvrA* at concentrations of up to 3200 µg/plate (Yoo, 1986).

In the Rec-assay, borneol [FL-no: 02.016] was reported to induce growth inhibition in *Bacillus subtilis* strain M45- when tested at concentrations of up to 10 mg/disc (Yoo, 1986). This test has very limited relevance for the genotoxicity evaluation.

In vivo

The potential of isobornyl propionate [FL-no: 09.131] to induce sex-linked recessive lethal mutations in adult *Drosophila melanogaster* was studied in a Basc test. No increased frequency of mutation was observed in flies fed with isobornyl propionate [FL-no: 09.131] in a 10 mmol/l solution for 3 days (Wild et al., 1983).

In the test for micronucleus formation, groups of NMRI mice given isobornyl propionate [FL-no: 09.131] at a dose of 841, 1893 or 2944 mg/kg body weight (bw) by intraperitoneal administration showed no increase in micronucleated erythrocytes in samples of bone marrow, 30 hours after administration (Wild et al., 1983).

Conclusion on genotoxicity

The testing of these representative bicyclic secondary alcohols, ketones and related esters in bacterial (Ames assay) and mammalian (micronucleus formation) in vivo systems showed no evidence of genotoxic potential, and these results are further supported by the lack of positive findings in the Drosophila Basc test. These data are supported by the lack of genotoxic potential of the related α,β-unsaturated monocyclic ketones, isophorone [FL-no: 07.126] and *d*-carvone [FL-no: 07.146] and *l*-carvone [FL-no: 07.147]. These substances were evaluated by JECFA to be of no safety concern but have also been evaluated by EFSA in FGE.212Rev2 to be of no concern with respect to genotoxicity (see section 4.4).

For a summary of *in vitro/in vivo* genotoxicity data considered by the JECFA, see Table 3.

4.2. Genotoxicity Studies - Text Taken[8] from EFSA FGE.47Rev1 (EFSA CEF Panel, 2012)

No *in vitro / in vivo* genotoxicity data are available for the candidate substances in FGE.47.

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

4.3. Genotoxicity Studies - Text Taken from FGE.211 (EFSA CEF Panel, 2011)

The following text is taken from FGE.211 and is relevant for the evaluation of pinocarveol [FL no: 02.100], which was one of the four substances in subgroup 2.5 of FGE.19 (FGE.211) for which a conclusion of no concern for genotoxicity was reached.

The Industry has submitted data concerning genotoxicity studies for a representative substance for this subgroup 2.5 of FGE.19, 1(7),8-*p*-menthadien-2-yl acetate [FL-no: 09.930] (structurally related to 1(7),8-*p*-menthadien-2-one).

In vitro data

The newly available data comprise a bacterial reverse mutation assay and an *in vitro* micronucleus assay with human peripheral blood lymphocytes. The genotoxicity assays have been performed on a commercial mixture of the representative substance 1(7),8-*p*-menthadien-2-yl acetate and a positional isomer, carvyl acetate. Carvyl acetate can be hydrolysed following oxidation to carvone, which has been evaluated by EFSA in FGE.212 (EFSA, 2009) and NTP (NTP, 1990) as non-genotoxic. The highest concentration of *d*-carvone that could be tested without cytotoxicity was 333 µg/plate (Mortelmans et al., 1986), i.e. the cytotoxicity was in the same range as observed for the mixture of 1(7),8-*p*-menthadien-2-yl acetate/carvyl acetate. The Panel concluded that testing the commercial mixture of 1(7),8-*p*-menthadien-2-yl acetate/carvyl acetate for genotoxicity allows the evaluation of the genotoxic potential of 1(7),8-*p*-menthadien-2-yl acetate. The concentrations reported in Table 3 (in FGE.211) are for the mixture of substances.

Bacterial Reverse Mutation Assay

1(7),8-p-menthadien-2-yl acetate/carvyl acetate was tested for mutagenic activity according to OECD guideline 471 and in compliance with GLP (Beevers, 2010). The test material exhibited a marked toxicity as indicated by thinning of the background lawn, reduced revertant counts and complete killing of test bacteria. However, the Panel considered the remaining number of concentrations without signs of toxicity sufficient to draw a conclusion on mutagenicity in this system (for details see FGE.211 Table 3).

Overall, the Panel concluded that there was no evidence of mutagenic activity of 1(7),8-*p*-menthadien-2-yl acetate/carvyl acetate at concentrations up to those causing bactericidal effects.

In vitro Micronucleus Test

1(7),8-p-menthadien-2-yl acetate/carvyl acetate was tested for induction of micronuclei in human peripheral blood lymphocytes according to OECD guideline 487 and in compliance with GLP (Whitwell, 2010). The Panel considered that acceptable levels of cytotoxicity as judged upon the replication index were achieved at the top concentrations (for details see FGE.211 Table 3).

Overall, the Panel concluded that there was no evidence of chromosomal damage or aneuploidy, as evidenced by no increase in levels of micronucleated binucleate cells (MNBN) in the presence or absence of S9 metabolic activation.

Discussion of Mutagenicity/Genotoxicity Data

The commercial mixture of the representative substance 1(7),8--*p*- -menthadien-2-yl acetate and a positional isomer, carvyl acetate was tested for all three genetic endpoints: gene mutations, structural and numerical chromosomal aberrations. The test material did not induce gene mutations in bacteria and was not clastogenic and/or aneugenic in mammalian cells *in vitro*. Although this commercial mixture was cytotoxic at high concentrations the remaining concentrations without signs of toxicity provide a valid data set.

Conclusion

The *in vitro* genotoxicity data on the commercial mixture of the representative substance 1(7),8-*p*-menthadien-2-yl acetate [FL-no: 09.930] and a positional isomer, carvyl acetate do not indicate genotoxic potential. Accordingly the four substances in this subgroup 2.5 of FGE.19 (FGE.211) would be of no safety concern with respect to genotoxicity, and will then be evaluated through the Procedure.

4.4. Genotoxicity Studies - Text Taken from FGE.212Rev2 (EFSA CEF Panel, 2014a)

The following text is taken from FGE.212Rev2 and is relevant for the evaluation of pin-2-en-4-ol [FL no: 02.101], which was one of the isophorone-related substances in subgroup 2.6 of FGE.19 (FGE.212Rev1) for which a conclusion of no concern for genotoxicity was reached.

There are studies available for four substances in this FGE (FGE.212Rev2). For tetramethyl ethylcyclohexenone (mixture of isomers) [FL-no: 07.035] one *in vitro* and one *in vivo* study have been evaluated.

Seven *in vitro* and three *in vivo* studies are available for 3,5,5 trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone).

Three *in vitro* studies are available concerning *d*-carvone [FL-no: 07.146] and two *in vitro* studies concerning *l*-carvone [FL-no: 07.147].

Study validation and results are presented in Tables 5 and 6 of FGE.212Rev2.

3,5,5 Trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone) did not induce gene mutations in bacteria but it induced mutations in mammalian cells in a mouse lymphoma TK assay in the absence of metabolic activation (it was not tested in the presence of metabolic activation) (NTP, 1986). No mutations in the MLTK assay were observed in a study of O'Donoghue et al. (O'Donoghue et al., 1988) at comparable concentrations. Isophorone induced chromosomal aberrations in Chinese hamster lung fibroblasts with and without metabolic activation (Matsuoka et al., 1996) and sister chromatid exchanges (SCE) in CHO cells without metabolic activation (Gulati et al., 1989). Chromosomal aberrations have not been observed in two other studies (Gulati et al., 1989; NTP, 1986); however, the validity of the results was limited because the types of aberrations were not reported. Isophorone did not induce unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro*. *In vivo*, isophorone was tested negative in a sex-linked recessive lethal mutation assay in *Drosophila* (Foureman et al., 1994) and in two micronucleus assays in mice (McKee et al., 1987; O'Donoghue et al., 1988). However, the *Drosophila* assay has only limited relevance and the micronucleus assays were of limited validity.

Negative results were also observed with tetramethyl ethylcyclohexenone [FL-no: 07.035] in bacteria, in a sex-linked recessive lethal mutation assay in *Drosophila* (Wild et al., 1983) and in a mouse micronucleus assay (Wild et al., 1983); however, there was a mixture of isomers tested and the studies were only of limited validity.

d-Carvone [FL-no: 07.146] was not mutagenic in bacteria but induced SCE and chromosomal aberrations in CHO cells in the presence and absence of metabolic activation, respectively (NTP, 1990).

Conclusion on Genotoxicity and Carcinogenicity (cited from FGE.212Rev2)

The Panel concluded that 3,5,5 trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone) is genotoxic *in vitro* while a final conclusion on the genotoxicity *in vivo* could not be drawn based on the data available. It is carcinogenic in male rats and male mice. It was also predicted to be genotoxic in one of the four MultiCASE models (while it was out of domain in the ISS model).

d-Carvone [FL-no: 07.146] is genotoxic *in vitro* while no *in vivo* data were available. *d*-Carvone, was not carcinogenic in mice and was predicted to be non-genotoxic in the four MultiCASE models (while it was out of domain in the ISS model). No data are available on *l*-carvone. However, *in vivo* studies in humans show that the metabolism of ingestion-correlated amounts of *d*- or *l*-carvone occurs via a major oxidative pathway of the isopropylene side chain yielding diol and two carboxylic acids, irrespective of the stereochemical difference between the two parent isomers of carvone (Engel, 2001). Accordingly, the results for *d*-carvone can be used for *l*-carvone as well.

The negative results reported from *in vivo* studies on the genotoxicity of tetramethyl ethylcyclohexenone [FL-no: 07.035] were only of limited validity.

Data submitted from Industry in reply to Genotoxicity Data requested in FGE.212 (cited from the FGE.212Rev2)

Honma et al. (Honma et al., 1999a, b) found that isophorone did not clearly induce mutations in the mouse lymphoma assay (MLA) following 3 hour treatments, but observed that it was mutagenic after 24 hour treatments in the absence of S9. Although only graphs are plotted, it seems that increases in mutation frequency (MF) that exceeded the Global Evaluation Factor (GEF) occurred at around 1250-1500 µg/ml where toxicity (by relative survival) reached 70-90 %.

The NTP conducted a mouse bone marrow chromosomal aberration (CA) study on isophorone. Groups of eight male B6C3F1 mice (larger group sizes than required by OECD) were dosed i.p. with isophorone at 125, 250 and 500 mg/kg bw. The standard protocol for *in vivo* CA is not given on the NTP website. However, based on Shelby and Witt (Shelby and Witt, 1995), animals should have been sampled at 17 hour and, if negative, also at 36 hours. The data on the NTP website are only for bone marrow sampled at 36 hour. It is therefore possible that a 17 hours sample was also taken, and found to be negative, but the data have not been posted. Fifty cells per animal were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure. The control CA frequency was normal (2.75 %) and the positive control (dimethylbenzanthracene) produced a significant response in CA frequency.

A DNA binding study was conducted in which F344-rats and B6C3F1-mice (the strains used in the NTP carcinogenicity study) were exposed to isophorone (Thier et al., 1990). Animals of both sexes were dosed once or five times by gavage with 500 mg/kg bw of unlabelled isophorone spiked with [1,3,5-^{14}C]-isophorone (specific activity: 52 mCi per mmol, 1.92 GBq per mmol). An additional group of acute dosed male rats received undiluted ^{14}C-isophorone for increased sensitivity. Rats and mice were maintained for 24 hours in closed metabolic cages. Twenty four hours after exposure, livers and kidneys (the tumour target tissues) were removed from the animals. DNA was isolated through hydroxyapatite chromatography and radioactivity was measured by liquid scintillation counting. No positive controls were included. Also no untreated controls were included, but, except for the liver sample of one mouse in the five times dose group, radioactivity values were within 2σ of background (6 dpm). Radioactivity values therefore did not indicate significant attachment of radioactivity to DNA. From these results it can be concluded that neither isophorone nor its metabolites bind covalently to DNA.

A study (Morishita et al., 1997) was designed to investigate whether isophorone and/or α2µ-globulin[9] might be involved in the induction of preputial gland tumours in F-344 rats (10/sex/dose group). A series of experiments was performed in order to study several parameters including:

- Binding of isophorone to DNA of kidney and preputial gland. Groups of 10 male rats were dosed by gavage with 500 mg/kg of [^{14}C]-isophorone (specific activity 14.65 mCi/mmol; 100 µCi/animal). Positive control animals were dosed with ^{3}H-labeled methyl nitrosourea.

[9] Since interaction with α2µ-glubulin is not of direct relevance for the evaluation of genotoxic potential, this information is omitted from this study summary.

- DNA adduct detection by ^{32}P-postlabeling in young adult male and female rats (7 per group) dosed by gavage with 0, 250 or 500 mg/kg isophorone for five days.

Extraction of preputial gland and kidney DNA from rats treated with single 500 mg/kg labeled doses yielded no evidence of isophorone binding to DNA, whereas the positive control showed significant binding to DNA of preputial gland and kidney. These negative results with isophorone were confirmed in the ^{32}P -postlabeling assays.

In addition Industry has also asked whether the information submitted for isophorone, (cyclohexenyl derivative), could also be applied to evaluate the genotoxic potential of the five-carbon membered ring substances (i.e. cyclopentenyl derivatives) in subgroup 2.6 (letter of EFFA to EFSA, dated 14/4-2010) (EFFA, 2010b). This request was supported by the argumentation that there is structural resemblance with respect to steric hindrance around the α,β-unsaturated double bond. In addition, Industry argued that the π-conjugation systems in these molecules is very nearly planar and that therefore the reactivity and genotoxic potentials of the five- and six-membered ring systems would be similar. No further data were provided to substantiate this argumentation.

Discussion of the Additional Data (cited from the FGE.212Rev2)

Conflicting results were reported in two valid studies with the mouse lymphoma assay (MLA): one negative (O'Donoghue et al., 1988) and one positive (NTP, 1986) at comparable concentrations. Mixed results were also reported in two studies of limited validity: one negative (Honma et al., 1999a) and one positive (Honma et al., 1999b). Another negative result was reported in a study (McKee et al., 1987), the validity of which cannot be evaluated. In the light of the clearly negative results in two valid bacterial gene mutation tests (Ames test) and in a valid Sex Linked Recessive Lethal Mutations test (SLRL) in *Drosophila*, and taking into account the lack of specificity and high sensitivity of the MLA, overall the results presently available are considered of questionable relevance. The Panel agrees that isophorone demonstrates some genotoxic activity *in vitro* but that the new data demonstrate lack of clastogenicity *in vivo*. In addition, the new DNA-binding data from two separate studies provide convincing evidence that isophorone does not induce tumours via a genotoxic mechanism. On the basis of these data it may be argued that there is no need to perform further *in vivo* genotoxicity studies such as the Comet assay or bone marrow micronucleus test. Thus, based on the data available the Panel concluded that there is no concern with respect to genotoxicity of isophorone.

Since based on the additional information the concern for the genotoxic potential for isophorone has been alleviated, The Panel concluded in FGE.212Rev2 that a genotoxic potential could also be ruled out for the other isophorone-related six-carbon members of this subgroup of FGE.19.

Study validation and results are presented in Table 6 of FGE.212Rev2 (EFSA CEF Panel, 2014a).

4.5. Genotoxicity Studies - Text Taken from FGE.213Rev1 (EFSA CEF Panel, 2014b)

The following text is taken from FGE.213Rev1 and is relevant for the evaluation of nootkatone and 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.089 and 07.136], which are two of the 13 substances in subgroup 2.7 of FGE.19 (FGE.213Rev1) for which a conclusion of no concern for genotoxicity was reached.

Bacterial Reverse Mutation Assay

Nootkatone [FL-no: 07.089] was tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence or presence of S9-mix (Marzin, 1998). A preliminary toxicity test to identify appropriate concentrations for the mutagenicity assays was performed in the absence and presence of S9-mix, and cytotoxicity was observed at 50 µg/plate in the absence of S9-mix and at 150 µg/plate in the presence of S9-mix. In the first mutagenicity experiment using plate incorporation methodology the concentrations tested were 0.5, 1.5, 5, 15, and 50 µg/plate in the absence of S9-mix

metabolic activation, and 1.5, 5, 15, 50, and 150 µg/plate in the presence of S9-mix. In the second experiment the pre-incubation method was used and the concentrations were 0.5, 1.5, 5, 15, and 50 µg/plate in the absence of S9-mix metabolic activation, and 0.5, 1.5, 5, 15, 50, and 150 µg/plate in the presence of S9-mix. Thus, the study design complied with current recommendations and an acceptable top concentration was achieved. There was no evidence of any mutagenic effect induced by nootkatone in any of the strains, either in the absence or presence of S9-mix.

Micronucleus Assays

Nootkatone [FL-no: 07.089] 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 S9-mix fraction as an *in vitro* metabolising system (Stone, 2011b). Cells were stimulated for 48 hours with phytohaemagglutinin to produce exponentially growing cells, and then treated for 3 hours (followed by 21 hours recovery) with 0, 50, 70 or 80 µg/ml of nootkatone in the absence of S9-mix and 0, 160, 180 and 185 µg/ml in the presence of S9-mix, respectively. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 60 and 58 % respectively. In a parallel assay, cells were treated for 24 hours with 0, 10, 15, 22 and 24 µg/ml of nootkatone in the absence of S9-mix with no recovery period. The top concentration induced 62 % cytotoxicity. There were two replicate cultures per treatment, and 1000 binucleate cells per replicate (i.e. 2000 cells per dose) were scored for micronuclei. 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. No evidence of chromosomal damage or aneuploidy was observed as frequencies of MNBN cells were not significantly different from concurrent controls and fell within historical control ranges for all treatments with nootkatone in the presence or absence of S9-mix metabolic activation (Stone, 2011).

Study validation and results are presented in Table 8 of FGE.213Rev1 (EFSA CEF Panel, 2014b).

4.6. EFSA Considerations

For four of the candidate substances [FL-no: 02.100, 02.101, 07.089 and 07.136] it has been concluded in FGE.211, FGE.212Rev2 and FGE.213Rev1, respectively, that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances. Thus, the Panel concluded that these substances can be evaluated through the Procedure in FGE.87. For the remaining 15 candidate substances in FGE.87 [FL-no: 02.016, 02.038, 02.059, 07.153, 07.159, 09.017, 09.082, 09.131, 09.153, 09.176, 09.218, 09.269, 09.319, 09.456 and 09.457], genotoxicity data are available on two substances [FL-no: 02.016 and 09.131]. The genotoxic potential of these two substances could not be adequately assessed. However, the data available do not preclude the evaluation of these 15 candidate substances using the Procedure.

5. Application of the Procedure

5.1. Application of the Procedure to 19 Bicyclic Secondary Alcohols, Ketones and related Esters by the JECFA (JECFA, 2006a)

According to the JECFA, 15 of the 19 substances belong to structural class I and four to structural class II using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded the 19 bicyclic secondary alcohols, ketones and related esters at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and concluded that the intakes for all substances are below the thresholds for their structural classes I and II (step A3).

In conclusion, the JECFA considered that the bicyclic secondary alcohols, ketones and related esters evaluated through the Procedure, were of no safety concern at the estimated levels of intakes based on the MSDI approach.

The evaluations of the 19 bicyclic secondary alcohols, ketones and related esters are summarised in Table 4.

5.2. Application of the Procedure to Bicyclic Secondary Alcohols, Ketones and Related Esters by EFSA in FGE.47Rev1 (EFSA CEF Panel, 2012)

For the safety evaluation of the six candidate substances from chemical group 7 and 8 the Procedure as outlined in Annex I was applied, based on the MSDI approach.

Step 1

Four candidate substances are classified into structural class I [FL-no: 02.119, 09.584, 09.848 and 09.888] and two [FL-no: 07.171 and 07.196] into structural class II according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

All six candidate substances in this group are expected to be metabolised to innocuous products. The evaluation of these substances therefore proceeded via the A-side of the Procedure scheme.

Step A3

The candidate substances [FL-no: 02.119, 07.171, 07.196, 09.584, 09.848 and 09.888] have estimated European daily *per capita* intakes ranging from 0.011 to 34 µg. These intakes are below the threshold of concern of 1800 µg/person/day for structural class I and 540 µg/person/day for structural class II substances.

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

The stepwise evaluations of the six substances are summarised in Table 5.

5.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA for the bicyclic secondary alcohols, ketones and related esters. EFSA also agrees with the conclusion reached at step A3 of the Procedure for 18 substances in FGE.87 that they do not pose a safety concern when used as flavouring substances at estimated levels of intake, based on the MSDI approach. For the flavouring substance 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136] this conclusion could not be reached because no annual production volume for Europe is available for this substance. Therefore no exposure estimate (MSDI) can be calculated and the evaluation through the Procedure cannot be finalised.

CONCLUSION

The Panel concluded that all 19 substances in the JECFA flavouring group of bicyclic secondary alcohols, ketones and related esters are structurally related to the group of bicyclic secondary alcohols, ketones and related evaluated by EFSA in the Flavouring Group Evaluation 47, Revision 1 (FGE.47Rev1).

The Panel agrees with the application of the Procedure as performed by the JECFA for the bicyclic secondary alcohols, ketones and related esters. It was concluded at step A3 of the Procedure that 18 substances do not pose a safety concern when used as flavouring substances at estimated levels of intake, based on the MSDI approach. For the flavouring substance 4,4a,5,6-tetrahydro-7-methylnapthalen-2(3H)-one [FL-no: 07.136] this conclusion could not be reached and the evaluation

through the Procedure cannot be finalised, because no annual production volume for Europe is available for this substance, from which a MSDI value can be calculated.

For all 19 substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessments and to finalise the evaluation.

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. Specifications are available for all the materials of commerce, however, a production volume for EU for substance [FL-no: 07.136] is not available, which precludes the finalisation of the evaluation by EFSA of this substance.

For the remaining 18 JECFA–evaluated bicyclic secondary alcohols, ketones and related esters [FL-no: 02.016, 02.038, 02.059, 02.100, 02.101, 07.089, 07.153, 07.159, 09.017, 09.082, 09.131, 09.153, 09.176, 09.218, 09.269, 09.319, 09.456 and 09.457] the Panel agrees with the JECFA conclusion: “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

SUMMARY OF GENOTOXICITY DATA

Table 3: Genotoxicity Data (*in vitro / in vivo*) JECFA (JECFA, 2006a)

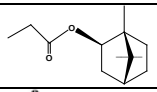
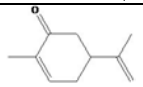
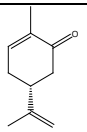
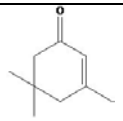
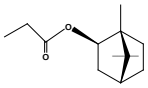
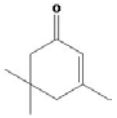
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
02.016 1385	Borneol		Reverse mutation	*S. typhimurium* TA97, TA98, TA100	1 mg/ml (1000 µg/ml)	Negative[1]	(Azizan and Blevins, 1995)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤ 5 mg/plate (5000 µg/plate)	Negative[1]	(Simmon et al., 1977)
			DNA repair	*B. subtilis* M45$^-$ and H17$^+$	≤ 10 mg/disc	Positive	(Yoo, 1986)
			Mutation test	*E. coli* WP2 *uvr*A (trp-)	0.4-3.2 mg/plate	Negative	(Yoo, 1986)
09.131 1391	Isobornyl propionate		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤ 3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)
07.012 380	Carvone		Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative	(Florin et al., 1980)
			DNA repair	*B. subtilis* M45 (rec$^-$) and H17 (rec$^+$)	0.6 ml/disc	Negative	(Matsui et al., 1989)
07.147 380.2	*l*-Carvone		Gene mutation (preincubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	333 µg/plate	Negative	(Mortelmans et al., 1986)
			Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	333 µg/plate	Negative	(NTP, 1990)
			Sister chromatid exchange	Chinise hamster ovary cells	502 µg/plate	Equivocal	(NTP, 1990)
			Chromosomal aberration	Chinise hamster ovary cells	400 µg/plate	Equivocal	(NTP, 1990)
07.126 1112	Isophorone		Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	33 - 10000 mg/plate	Negative[1]	(Mortelmans et al., 1986)
			Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33 - 10000 mg/plate	Negative[1]	(NTP, 1986)
			Mutation	L5178YTk$^{+/-}$ mouse	67 - 810 mg/ml	Negative[4]	(McKee et al., 1987)

Table 3: Genotoxicity Data (*in vitro / in vivo*) JECFA (JECFA, 2006a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
				lymphoma cells			
					130 - 1300	Negative[5]	(McKee et al., 1987)
			Mutation	L5178YTk$^{+/-}$ mouse lymphoma cells	0.089 - 0.89 mg/ml	Negative[4]	(O'Donoghue et al., 1988)
						Negative[5]	(O'Donoghue et al., 1988)
			Mutation	L5178YTk$^{+/-}$ mouse lymphoma cells	800 mg/ml[6]	Positive[5]	(McGregor et al., 1988)
			Mutation	L5178YTk$^{+/-}$ mouse lymphoma cells	1200 mg/ml	Negative[4]	(NTP, 1986)
						Positive[5]	
			Chromosomal aberration	Chinese hamster ovary cells	5 – 1600 mg/ml	Negative[1]	(Gulati et al., 1989)
						Positive[4,7]	
			Chromosomal aberration	Chinese hamster lung fibroblasts	1200[5], 1500[4] mg/ml	Positive[1]	(Matsuoka et al., 1996)
					250 – 1000 mg/ml	Negative[1]	
			Sister chromatid exchange	Chinese hamster ovary cells	5 – 1600 mg/ml	Positive[4,7]	(Gulati et al., 1989)
			Sister chromatid exchange	Chinese hamster ovary cells	160 – 1000 mg/ml	Negative[1]	(NTP, 1986)
			Unscheduled DNA synthesis	Rat hepatocytes	0.005 – 0.4 ml/ml	Negative[1]	(O'Donoghue et al., 1988)
			Unscheduled DNA synthesis	Rat hepatocytes	200 ml/ml	Negative[1]	(McKee et al., 1987)
In vivo							
09.131 1391	Isobornyl propionate		Somatic mutation and recombination	*D. melanogaster*	10 mmol/l (2103 µg/ml)	Negative[2]	(Wild et al., 1983)
			Micronucleus formation	Mouse bone marrow cells	841, 1893 and 2944 mg/kg bw	Negative[3]	(Wild et al., 1983)
07.126 1112	Isophorone		Sex-linked recessive lethal mutation	*D. melanogaster*	2000[8] and 12500[3] ppm	Negative	(Foureman et al., 1994)
			Micronucleus formation	CD-1 mice	540 mg/kg bw	Negative	(McKee et al., 1987)
			Micronucleus formation	CD-1 mice	0.54 ml/kg bw	Negative	(O'Donoghue et al., 1988)

[1] Tested with and without metabolic activation.
[2] Dose calculated based on the relative molecular mass of substance = 210.32.
[3] Administered via intraperitoneal injection.
[4] Without metabolic activation.
[5] With metabolic activation.
[6] Cytotoxic at next highest dose tested (1600 mg/ml).
[7] A positive response was obtained only in the absence of metabolic activation and only after additional culture time (6–13 h).
[8] Oral administration.

No *in vitro / in vivo* genotoxicity data are available for the candidate substances in FGE.47Rev1 (EFSA CEF Panel, 2012).

SUMMARY OF SAFETY EVALUATIONS

Table 4: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

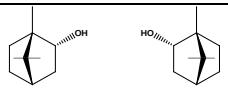
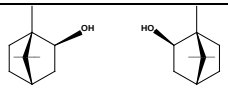
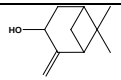
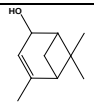
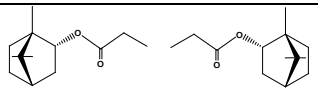
FL-no JECFA -no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.016 1385	Borneol		130 23	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Borneol. No safety concern at the estimated level of intake based on the MSDI approach.
02.038 1397	Fenchyl alcohol		55 17	Class I A3: Intake below threshold	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.
02.059 1386	Isoborneol		21 0.07	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Isoborneol. No safety concern at the estimated level of intake based on the MSDI approach.
02.100 1403	Pinocarveol		0.012 0.01	Class I A3: Intake below threshold	d	Evaluated in FGE.211, genotoxicity concern could be ruled out.	No safety concern at the estimated level of intake based on the MSDI approach.
02.101 1404	Pin-2-en-4-ol		0.012 0.2	Class I A3: Intake below threshold	d	Evaluated in FGE.212Rev1, genotoxicity concern could be ruled out.	No safety concern at the estimated level of intake based on the MSDI approach.
09.017 1387	Bornyl acetate		18 3	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Bornyl acetate. No safety concern at the estimated level of intake

Table 4: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

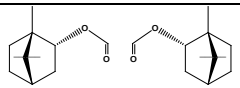
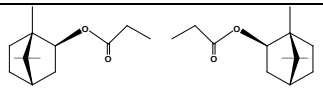
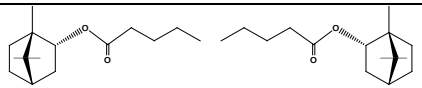
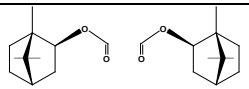
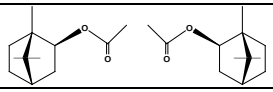
FL-no JECFA -no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
							based on the MSDI approach.
09.082 1389	Bornyl formate		1.2 0.09	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Bornyl formate. No safety concern at the estimated level of intake based on the MSDI approach.
09.131 1391	Isobornyl propionate		2.6 0.007	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Isobornyl propionate. No safety concern at the estimated level of intake based on the MSDI approach.
09.153 1392	Bornyl valerate		3.7 5	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Bornyl valerate. No safety concern at the estimated level of intake based on the MSDI approach.
09.176 1390	Isobornyl formate		0.61 0.4	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Isobornyl formate. No safety concern at the estimated level of intake based on the MSDI approach.
09.218 1388	Isobornyl acetate		890 236	Class I A3: Intake below	d	No safety concern at the estimated level of intake based on the MSDI	Register name to be changed to *DL*-Isobornyl acetate.

Table 4: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

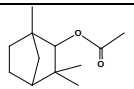
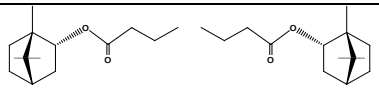
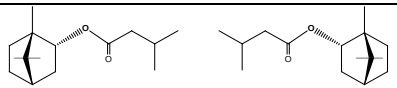
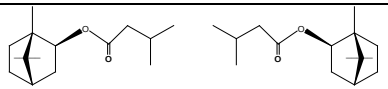
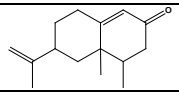
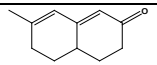
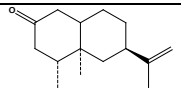
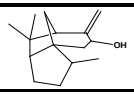
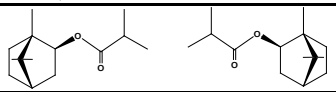
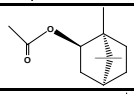
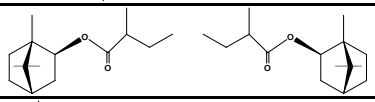
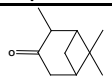
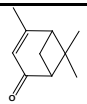
FL-no JECFA -no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
				threshold		approach.	No safety concern at the estimated level of intake based on the MSDI approach.
09.269 1399	Fenchyl acetate		2.9 0.07	Class I A3: Intake below threshold	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.
09.319 1412	Bornyl butyrate		6.1 9	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	CASrn in Register refers to (1R,2S,4R)-rel. Register name to be changed to *DL*-Bornyl butyrate. No safety concern at the estimated level of intake based on the MSDI approach.
09.456 1393	Bornyl isovalerate		0.12 0.5	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Bornyl isovalerate. No safety concern at the estimated level of intake based on the MSDI approach.
09.457 1394	Isobornyl isovalerate		0.012 0.08	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to *DL*-Isobornyl isovalerate. No safety concern at the estimated level of intake based on the MSDI approach.
07.089 1398	Nootkatone		130 20	Class II A3: Intake below	d	Evaluated in FGE.213Rev1, genotoxicity concern	No safety concern at the estimated level of intake based on the MSDI

Table 4: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

FL-no JECFA -no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/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
				threshold		could be ruled out.	approach.
07.136 1405	4,4a,5,6-Tetrahydro-7-methylnapthalen-2(3H)-one		0.04	Class II A3: Intake below threshold	d	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out.	EU production volume is not available. This precludes the finalisation of the evaluation.
07.153 1407	1,10-Dihydronootkatone		0.24 0.9	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	CASrn refers to (4R,4aS,6R,8aS)-stereoisomer. Register name to be changed accordingly. No safety concern at the estimated level of intake based on the MSDI approach.
07.159 1396	*d*-Fenchone		6.3 5	Class II A3: Intake below threshold	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.

(a): EU MSDI: Amount added to food as flavour in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita/day.
(b): Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/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 5: Summary of Safety Evaluation by the EFSA (FGE.47Rev1) (EFSA CEF Panel, 2012)

FL-no	EU Register name	Structural formula	MSDI [a] (µg/*capita*/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
02.119	Cedrenol		34	Class I A3: Intake below threshold	d	f	i
09.584	Isobornyl isobutyrate		0.085	Class I A3: Intake below threshold	d	f	
09.848	(1*S*-endo)-1,7,7-Trimethylbicyclo[2.2.1] heptan-2-ol acetate		0.011	Class I A3: Intake below threshold	d	f	
09.888	Isobornyl 2-methylbutyrate		0.061	Class I A3: Intake below threshold	d	f	
07.171	Isopinocamphone		0.024	Class II A3: Intake below threshold	d	f	
07.196	Pin-2-en-4-one		15	Class II A3: Intake below threshold	d	f	j

(a) EU MSDI: Amount added to food as flavour in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/*capita*/day.
(b) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/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.
(i) Evaluated in FGE.211, genotoxicity concern could be ruled out.
(j) Evaluated in FGE.212Rev1, genotoxicity concern could be ruled out.

DOCUMENTATION PROVIDED TO EFSA

1. Beevers C, 2010. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. 1(7),8-*p*-menthadien-2-yl acetate. Covance Laboratories Ltd, England. Study no. 8213039. April 2010. Unpublished report submitted by EFFA to FLAVIS Secretariat.

2. EFFA (European Flavour Association), 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

3. EFFA (European Flavour Association), 2010b. EFFA letter to EFSA/Commission concerning: Submission of additional data related to FGE.19 subgroup 2.6. Dated 14 April 2010. FLAVIS/8.142.

4. EFFA (European Flavour Association), 2011. E-mail from EFFA/DG SANCO to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark. Dated 14 September 2011. FGE.87 concerning correct chemical names. FLAVIS/8.133.

5. EFFA (European Flavour Association), 2014. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark. Dated 1 July 2014. Information on substances [FL-no: 02.100, 02.101, 07.089, 07.136] in FGE.87Rev2. FLAVIS/8.250.

6. Marzin D, 1998. Recherche de mutagenicite sur *salmonella typhimurium* his - selon la methode de B.N. Ames sur le produit ST14C97 [Bacterial reverse mutation assay of nootkatone (Ames test)]. Institut Pasteur de Lille. Rapport no. IPL-R980113/ST14C97/Firmenich Production. 29 Janvier 1998. Unpublished report submitted by EFFA to FLAVIS Secretariat. (In French)

7. Morishita K, Schoonhoven R and Swenberg JA, 1997. Mechanistic studies on isophorone and preputial gland carcinomas. Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, N.C. March 25, 1997. Unpublished report submitted by EFFA to FLAVIS secretariat.

8. Stone V, 2011. Induction of micronuclei in cultured human peripheral blood lymphocytes. Nootkatone. Covance Laboratories Ltd. Study no. 8242980. June, 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

9. Whitwell J, 2010. Induction of micronuclei in cultured human peripheral blood lymphocytes. 1(7),8-p-menthadien-2-yl acetate. Covance Laboratories Ltd, England. Study no. 822021. April 2010. Unpublished report submitted by EFFA to FLAVIS Secretariat.

REFERENCES

Azizan A and Blevins RD, 1995. Mutagenicity and antimutagenicity testing of six chemicals associated with the pungent properties of specific spices as revealed by the Ames Salmonella/microsomal assay. Archives of Environmental Contamination and Toxicology 28, 248-258.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetics Toxicology 16(3), 255-276.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on Flavouring Group Evaluation 211 (FGE.211): Consideration of genotoxicity data on representatives for one a,ß-unsaturated ketone and three precursors from chemical subgroup 2.5 of FGE.19 by EFSA. EFSA Journal 2011;9(3):1993, 13 pp. doi:10.2903/j.efsa.2011.1993

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012. Scientific Opinion on Flavouring Group Evaluation 47, Revision 1 (FGE.47Rev1): Bicyclic secondary alcohols, ketones and related esters from chemical group 8. EFSA Journal 2012;10(3):2637, 43 pp. doi:10.2903/j.efsa.2012.2637

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014a. Scientific Opinion on Flavouring Group Evaluation 212, Revision 2 (FGE.212Rev2): α,ß-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19. EFSA Journal 2014;12(2):3584, 43 pp. doi:10.2903/j.efsa.2014.3584

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014b. Scientific Opinion on Flavouring Group Evaluation 213, Revision 1 (FGE.213Rev1): α,ß-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19. EFSA Journal 2014;12(5):3661, 46 pp. doi:10.2903/j.efsa.2014.3661

EFSA (European Food Safety Authority), 2008a. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food related to Flavouring Group Evaluation 87: Consideration of bicyclic secondary alcohols, ketones and related esters evaluated by JECFA (63rd meeting) structurally related to bicyclic secondary alcohols, ketones and related esters evaluated by EFSA in FGE.47 (2008) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal 2008, 746, 1-22.

EFSA (European Food Safety Authority), 2008b. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Parma on 27 - 29 November 2007. Parma, 7 January 2008. [Online]. Available: http://www.efsa.europa.eu/EFSA/Event_Meeting/afc_minutes_26thplen_en.pdf.

EFSA (European Food Safety Authority), 2008c. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission on camphor in flavourings and other food ingredients with flavouring properties. The EFSA Journal 2008, 729, 1-15.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food related to Flavouring Group Evaluation 212 (FGE.212): a,ß-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal 2009, 878, 1-28.

EFSA (European Food Safety Authority), 2010. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food related to Flavouring Group Evaluation 95 (FGE.95): Consideration of aliphatic, linear or branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters evaluated by JECFA (69th meeting) structurally related to esters of branched- and straight-chain aliphatic saturated primary alcohols and of one secondary alcohol, and branched- and straight-chain unsaturated carboxylic acids evaluated by EFSA in FGE.05Rev1 (2008) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). EFSA Journal 2010;8(11):1207, 51 pp. doi:10.2903/j.efsa.2010.1207

Engel W, 2001. *In vivo* studies on the metabolism of the monoterpenes S-(+) - and R-(-)-carvone in humans using the metabolism of ingestion-correlated amounts (MICA) approach. Journal of Agricultural and Food Chemistry 49, 4069-4075.

Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 18, 219-232.

Foureman P, Mason JM, Valencia R and Zimmering S, 1994. Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environmental and Molecular Mutagenesis 23, 208-227.

Gulati DK, Witt K, Anderson B, Zeiger E and Shelby M, 1989. Chromosome aberration and sister-chromatid exchange test in Chinese hamster ovary cells *in vitro* III: Results with 27 chemicals. Environmental and Molecular Mutagenesis 13, 133-193.

Honma M, Hayashi M, Shimada H, Tanaka N, Wakuri S, Awogi T, Yamamoto KI, Kodani N-U, Nishi Y, Nakadate M and Sofuni T, 1999a. Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the *in vitro* chromosomal aberration test. Mutagenesis 14(1), 5-22.

Honma M, Zhang L-S, Sakamoto H, Ozaki M, Takeshita K, Momose M, Hayashi M and Sufuni T, 1999b. The need for long-term treatment in the mouse lymphoma assay. Mutagenesis 14(1), 23-29.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005a. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005b. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006a. Safety evaluation of certain food additives and contaminants. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006b. Sixty-seventh Meeting. Rome, 20-29 June 2006, Summary and Conclusions. Issued 7 July 2006.

Matsui S, Yamamoto R and Yamada H, 1989. The *Bacillus subtilis*/Microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Water Science & Technology 21, 875-887.

Matsuoka A, Yamakage K, Kusakabe H, Wakuri S, Asakura M, Noguchi T, Sugiyama T, Shimada H, Nakayama S, Kasahara Y, Takahashi Y, Miura KF, Hatanaka M, Ishidate M, Morita T, Watanabe K, Hara M, Odawara K, Tanaka N, Hayashi M and Sofuni T, 1996. Re-evaluation of chromosomal aberration induction on nine mouse lymphoma assay 'unique positive' NTP carcinogens. Mutation Research 369, 243-252.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C and Caspary WJ, 1988. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environmental and Molecular Mutagenesis 12, 85-153.

McKee RH, Phillips RD, Lerman SA, Slesinski RS, Rogers-Back AM, Curren RD and Putman DL, 1987. The genotoxic potential of isophorone. Environmental Mutagenesis 9(8), 71.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E, 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. Environmental and Molecular Mutagenesis 8(Suppl. 7), 1-119.

NTP (National Toxicology Program), 1986. Toxicology and carcinogenesis studies of isophorone (CAS no. 78-59-1) in F344/N rats and B6C3F1 mice (gavage studies). NTP-TR 291. NIH Publication no. 86-2547.

NTP (National Toxicology Program), 1990. Toxicology and carcinogenesis studies of *d*-carvone (CAS. no. 2244-16-8) in B6C3F1 mice (gavage studies). February 1990. NTP-TR 381. NIH Publication no. 90-2836.

O'Donoghue JL, Haworth SR, Curren RD, Kirby PE, Lawlor T, Moran EJ, Phillips RD, Putnam DL, Rogers-Back AM, Slesinski RS and Thilagar A, 1988. Mutagenicity studies on ketone solvents: Methyl ethyl ketone, methyl isobutyl ketone, and isophorone. Mutation Research 206, 149-161.

SCF (Scientific Committee for Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Shelby MD and Witt KL, 1995. Comparison of results from mouse bone marrow chromosome aberration and micronucleus test. Environmental and Molecular Mutagenesis 25, 302-313.

Simmon VF, Kauhanen K and Tardiff RG, 1977. Mutagenic activity of chemicals identified in drinking water. In: Scott D, Bridges BA and Sobels FH (Eds.). Progress in genetic toxicology. Elsevier/North-Holland Biomedical Press, pp. 249-258.

Thier R, Peter H, Wiegland HJ and Bolt HM, 1990. Letter to the Editors. DNA binding study of isophorone in rats and mice. Archives of Toxicology 64, 684-685.

Wild D, King MT, Gocke E and Eckhard K, 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food and Chemical Toxicology 21(6), 707-719.

Yoo YS, 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka City Medical Journal 34(3-4), 267-288.

ABBREVIATIONS

BW	Body weight
CAS	Chemical Abstracts Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practise
GC-MS	Gas chromatography-mass spectrometry
ID	Identity
I.p.	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCR	Polymerase Chain Reaction
SCE	Sister chromatic exchange

SCF	Scientific Committee on Food
UDS	Unscheduled DNA synthesis
WHO	World Health Organization