EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2016. Scientific Opinion on Flavouring Group Evaluation 51, Revision 2 (FGE.51Rev2): Consideration of alicyclic ketones and secondary alcohols and related esters evaluated by the JECFA (59th meeting) structurally related to alicyclic ketones secondary alcohols and related esters in FGE.09Rev6 (2015b)

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Flavouring Group Evaluation 51, Revision 2 (FGE.51Rev2): Consideration of alicyclic ketones and secondary alcohols and related esters evaluated by JECFA (59th meeting) structurally related to alicyclic ketones secondary alcohols and related esters in FGE.09Rev6 (2015)

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

Abstract

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) 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 (JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. 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 present consideration concerns a group of 24 alicyclic ketones and secondary alcohols and related esters evaluated by JECFA (59th meeting in 2002 and 63rd meeting in 2004). This revision is made due to inclusion of four additional substances cleared for genotoxicity concern compared to the previous version [FL-no: 07.033, 07.094, 07.112 and 07.140]. The Panel concluded for 23 substances that these do not give rise to safety concerns at the levels of dietary intake, estimated on the basis of the MSDI approach [FL-no: 02.209, 07.034, 07.035, 07.045, 07.094, 07.095, 07.098, 07.112, 07.126, 07.129, 07.140, 07.148, 07.149, 07.172, 07.179, 07.180, 07.257, 09.027, 09.140, 09.160, 09.230, 09.464 and 09.930]. However, for all substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have been considered and are adequate for 21 substances. For [FL-no: 07.094 and 07.112], information on the solubility in water and ethanol is missing. The chemical identity could not be unambiguously confirmed for substance [FL-no: 07.033]. Therefore the Panel could not consider the JECFA evaluation of this substance and information as to its chemical identity should be submitted.

Keywords: alicyclic ketones, JECFA 59th meeting, alicyclic secondary alcohols, FGE.09, FGE.212.

Requestor: European Commission

Question number: EFSA-Q-2015-00315, EFSA-Q-2015-00316, EFSA-Q-2015-00317 and EFSA-Q-2015-00319.

Correspondence: fip@efsa.europa.eu

Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Wim Mennes, Maria Rosaria Milana, André Penninks, Maria de Fátima Tavares Poças, Vittorio Silano, Andrew Smith, Christina Tlustos, Detlef Wölfle, Holger Zorn and Corina-Aurelia Zugravu.

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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 a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (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.

The present revision of FGE.51, FGE.51Rev2, is due to new genotoxicity data evaluated in FGE.212Rev3 (EFSA CEF Panel, 2015a), which deals only with the genotoxic potential of α, β-unsaturated flavouring substances. Based on these data, the Panel concluded that the data available could rule out the concern for genotoxicity for [FL-no: 07.033, 07.094, 07.112 and 07.140] and accordingly these substances can be evaluated through the Procedure in this revision. Three of these substances were evaluated by the JECFA at its 59th meeting in 2002 [FL-no: 07.033, 07.094 and 07.112] and the fourth was evaluated by the JECFA at its 63rd meeting. Since in the previous version of this FGE (FGE.51Rev1) 20 substances were discussed, the present revision will address 24 flavouring substances.

The Panel concluded that the 24 substances in the JECFA flavouring groups of alicyclic ketones, secondary alcohols and related esters and monocyclic and bicyclic secondary alcohols, ketones and related esters are structurally related to the group of secondary alicyclic saturated and unsaturated alcohols, ketones and esters with secondary alicyclic alcohol moieties evaluated by EFSA in Flavouring Group Evaluation 09, Revision 6 (FGE.09Rev6) (EFSA CEF Panel, 2015b).

The chemical identity of [FL-no: 07.033] could not be unambiguously confirmed. Therefore the Panel could not consider the JECFA evaluation of this substance. The current revision of FGE.51 will consider only the safety of 23 JECFA-evaluated substances.

For all 23 substances considered in this FGE, the Panel concluded that either they did not raise a concern with respect to genotoxicity, or that concerns with respect to genotoxicity due to the presence of a structural alert for this could be ruled out, based on experimental data.

The Panel agrees with the application of the Procedure as performed by the JECFA for the 23 substances considered in this FGE.

For 23 JECFA evaluated alicyclic ketones, secondary alcohols and related esters [FL-no: 02.209, 07.034, 07.035, 07.045, 07.094, 07.095, 07.098, 07.112, 07.126, 07.129, 07.140, 07.148, 07.149, 07.172, 07.179, 07.180, 07.257, 09.027, 09.140, 09.160, 09.230, 09.464 and 09.930] the Panel agrees with the JECFA conclusion 'no safety concern at estimated levels of intake as flavouring substance' based on the MSDI approach.

In order to determine whether the conclusion for the JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications: Adequate specifications including complete purity criteria and identity tests are available for 21 JECFA-evaluated substances. For [FL-no: 07.094 and 07.112] information on the solubility in water and ethanol is missing and therefore the conclusions on the named substance cannot be applied to the materials of commerce that correspond to these two FL-numbers. For substance [FL-no: 07.033] unambiguous information with respect to the chemical identity should be provided.

For all 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 assessment and to finalise the evaluation.

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1. Introduction

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

The use of flavouring is regulated under Regulation (EC) No 1334/2008[1] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of article 9(a) of this Regulation an evaluation and approval are required for flavouring substances.

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012.[2] The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000.[3]

On 25 November 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 212, Revision 1 (FGE.212Rev1): α, β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19.[4]

The Panel concluded that the argumentation of Industry to expand its conclusion for the six-carbon ring members of subgroup 2.6 also to the cyclopentenyl derivatives in this subgroup [FL-no: 07.033, 07.094, 07.112 and 07.140] was considered too limited, given the lack of support from experimental data. Therefore, additional genotoxicity tests are still required for the representative substance [FL-no: 07.112] already chosen by the Panel. Alternatively, a more thorough explanation (physico-chemical parameters; experimental underpinning) of the proposed similar reactivity of six- and five-membered ring substances should be provided by Industry.

The requested data have been submitted by the applicant.

In addition, the flavouring substance [FL-no: 07.219], trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, was put in FGE.212 (former FGE.19, subgroup 2.6b: α, β–unsaturated aldehydes and ketones and precursors) because of its structure relationship with this group. Although the substance as such is not mentioned in the data submitted by the applicant, the submitted data are likely to be relevant for [FL-no: 07.219] as well.

Therefore, this request covers as well the re-evaluation of trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219].

1.1.1. Terms of Reference as provided by the European Commission

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

1.2. Interpretation of the Terms of Reference

The additional flavouring substances, isojasmone [FL-no: 07.033], 3-methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one [FL-no: 07.094], 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] and 3-methyl-2-pentylcyclopent-2-en-1-one [FL-no: 07.140] were first allocated to FGE.212Rev2 for evaluation with respect to genotoxicity. Based on the new genotoxicity data submitted, the Panel concluded in FGE.212Rev3 that these four flavouring substances do not give rise to concern with respect to genotoxicity and can accordingly now be evaluated through the Procedure in FGE.51rev2.

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

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

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

[4] EFSA Journal 2011;9(3):1923

2. Data and Methodologies

2.1. Description of key aspects of the evaluation methodology

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 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.

2.1.1. 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, 2006).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a 'modified theoretical added maximum daily intake' (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by 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.

2.1.2. Threshold of 1.5 µg/person per day (step B5) used by the JECFA

The JECFA uses the threshold of concern of 1.5 µg/person per day as part of the evaluation procedure:

'The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram 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 forty-sixth 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.

2.1.3. 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.

2.1.4. 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.

2.1.5. 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.

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

At its 59th meeting the JECFA evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters. Two substances were not in the Register, and 10 are α, β-unsaturated ketones or precursors for such which have been considered together with other α, β-unsaturated substances. The remaining 13 flavouring substances have originally been considered by EFSA in the FGE.51 (EFSA, 2008a).

The first revision of FGE.51, FGE.51Rev1 included the consideration of seven additional substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] (EFSA CEF Panel, 2012). Six of these additional substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129 and 07.172] are α, β-unsaturated ketones originally allocated to FGE.211 and FGE.212. The seventh substance [FL-no: 09.930] is a precursor for such ketones originally allocated to FGE.211. The substances have been considered with respect to genotoxicity (EFSA CEF Panel, 2011a; 2011b) and the Panel concluded that the data available did rule out the concern for genotoxicity and accordingly the substances can be evaluated through the Procedure. Since the publication of FGE.51, the EU production volume has been provided for the substance, [FL-no: 09.230] for which the evaluation could not be finalised in the previous version of this FGE, due to lack of these data. Based on the submitted EU production volume the substance was evaluated in FGE.96[5] (EFSA CEF Panel, 2011c), but the information was also included in FGE.51. Finally, information on the stereoisomeric composition were provided for four substances [FL-no: 02.209, 07.045, 07.095 and 07.257] and composition of mixture for one substance [FL-no: 07.095] since the previous version of FGE.51 (EFFA, 2012). A search in open literature for the seven new substances did not provide any further data on toxicity or metabolism.

The present revision of FGE.51, FGE.51Rev2 includes the consideration of four additional substances isojasmone [FL-no: 07.033], 3-methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one [FL-no: 07.094], 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] and 3-methyl-2-pentylcyclopent-2-en-1-one [FL-no: 07.140].

[5] Consideration of 88 flavouring substances considered by EFSA for which EU production volumes / anticipated production volumes have been submitted on request by DG SANCO.

Table 1: Revisions of FGE.51

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.51	16 May 2007	http://www.efsa.europa.eu/en/efsajournal/doc/855.pdf	13
FGE.51Rev1	22 March 2012	http://www.efsa.europa.eu/en/efsajournal/doc/2636.pdf	20
FGE.51Rev2	3 December 2015	http://www.efsa.europa.eu/en/efsajournal/doc/4338.pdf	24

The substances have been considered with respect to genotoxicity in FGE.212 Revision 3 (EFSA CEF Panel, 2015a) and the Panel concluded that the data available did rule out the concern for genotoxicity and accordingly the substances can be evaluated through the Procedure. A search in open literature for these four substances did not provide any further data on toxicity or metabolism.

2.3. Presentation of the substances in the JECFA flavouring group

2.3.1. Description

Status

The JECFA has evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters at the 59th meeting in 2002 (JECFA, 2005) and a group of 32 monocyclic and bicyclic secondary alcohols, ketones and related esters at the 63rd meeting in 2004 (JECFA, 2005).

EFSA Considerations

From the group evaluated by JECFA at its 59th meeting in 2002, two of the JECFA-evaluated substances are not in the Register (4-methyl cyclohexanone (JECFA no: 1104) and (E)-2-(2-octenyl) cyclopentanone (JECFA no: 1116)).

Of the remaining 23 substances, ten are α, β-unsaturated ketones or precursors for such. Seven of these [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] have been considered with respect to genotoxicity in FGE.211 (EFSA CEF Panel, 2011a) and FGE.212Rev1 (EFSA CEF Panel, 2011b), and the Panel concluded that the data available did rule out the concern for genotoxicity and accordingly the seven substances can be evaluated through the Procedure. For the remaining three α, β–unsaturated substances [FL-no: 07.033, 07.094 and 07.112] considered with respect to genotoxicity in FGE.212Rev1, a final conclusion of genotoxic properties could not be reached and additional data were requested. These data were submitted and evaluated in FGE.212Rev3 (EFSA CEF panel, 2015a) and the Panel concluded that the data available did rule out the concern for genotoxicity and accordingly the three substances can be evaluated through the Procedure.

From the group evaluated by JECFA at its 63rd meeting in 2004, three of the JECFA-evaluated substances are not in the Register (α-isomethylionyl acetate (JECFA no: 1410), d,l-Menthol(±)-propylene glycol carbonate (JECFA no: 1413) and l-Monomenthyl glutarate (JECFA no: 1414)). Of the remaining 29 JECFA evaluated substances, 25 substances are considered by EFSA in other FGEs (in FGE.56, FGE.56, FGE.73 and FGE.87), one is evaluated in a separate EFSA Opinion (EFSA, 2008b) and two are no longer supported by Industry as flavouring substances in Europe (cycloheptadec-9-en-1-one (JECFA no: 1401, former FL-no: 07.110) and 3-methylcyclopentadecan-1-one (JECFA no: 1402, former FL-no: 07.111). The one remaining substance, [FL-no: 07.140] was considered with respect to genotoxicity in FGE.212Rev3 (EFSA CEF panel, 2015a) and the Panel concluded that the data available did rule out the concern for genotoxicity. Accordingly the substance can be evaluated through the Procedure.

This consideration will therefore deal with 24 JECFA-evaluated substances.

The Panel concluded that the 24 substances in the JECFA flavouring groups of alicyclic ketones, secondary alcohols and related esters and monocyclic and bicyclic secondary alcohols, ketones and related esters are structurally related to the group of secondary alicyclic saturated and unsaturated

alcohols, ketones and esters with secondary alicyclic alcohol moieties evaluated by EFSA in Flavouring Group Evaluation 09, Revision 6 (FGE.09Rev6) (EFSA CEF Panel, 2015b).

2.3.2. Isomers

Status

Six of the substances have one chiral centre [FL-no: 07.045, 07.129, 07.172, 07.179, 07.180 and 07.257] and four substances have two or more chiral centres [FL-no: 02.209, 07.035, 07.095 and 09.930]. Three substances have possibility for *cis*/*trans* isomerism [FL-no: 07.034, 07.094 and 07.257]. For substance [FL-no: 07.033] originally information was provided that this substance consists of a mixture of isomers (see Table 2). Upon a request for further information with respect to the precise chemical composition and configuration of this material additional information was submitted (EFFA, 2015). However, this additional information did not result in a better characterisation of [FL-no: 07033]. Therefore the Panel concluded that for substance [FL-no: 07.033] the information on identity and composition is inadequate (e.g. chemical structures and CAS numbers provided are contradictory).

EFSA Considerations

Adequate information on isomeric composition is available for all substances, except for one substance, [FL-no: 07.033] for which the chemical identity cannot be unambiguously be stated.

2.3.3. Specifications

Status

The JECFA specifications are available for all 24 substances (JECFA, 2002). See Table 2.

EFSA Considerations

The available specifications are considered adequate for all substances, except for [FL-no: 07.033, 07.094 and 07.112]. For [FL-no: 07.094 and 07.112] information on the solubility in water and ethanol is missing. For substance [FL-no: 07.033] the chemical identity cannot be confirmed. As a consequence, the JECFA evaluation of [FL-no: 07.033] cannot be considered by the Panel.

2.3.4. Intake estimations

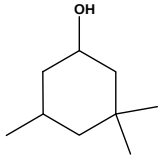
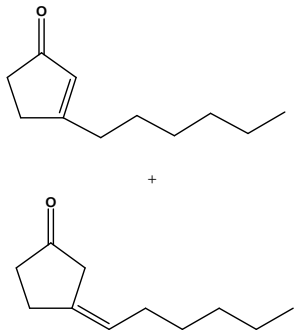
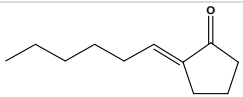
Status

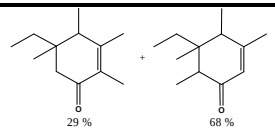
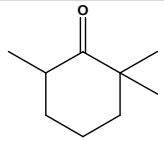
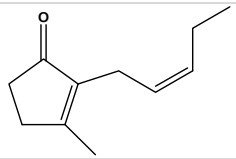
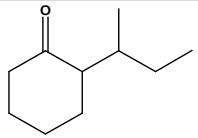
For all substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 11.

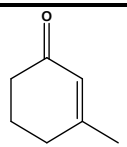
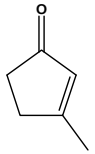
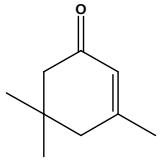
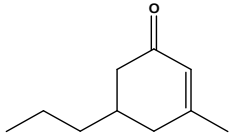
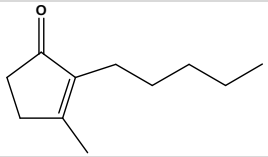
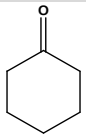
EFSA Considerations

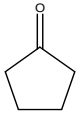
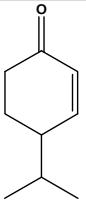
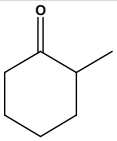
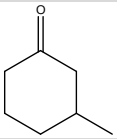
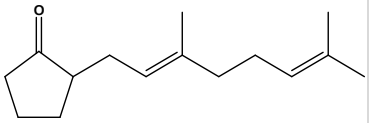
Tonnage data are available for the EU allowing calculation of the intake estimates (MSDI). The Panel noted that since no use levels were submitted, mTAMDI values cannot be calculated.

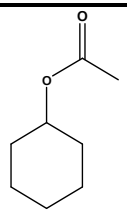
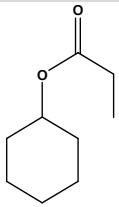
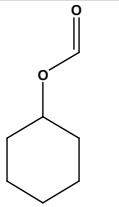
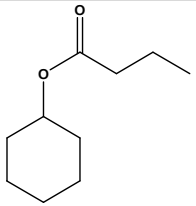
Table 2: Summary of specification data for substances evaluated by the JECFA (JECFA, 2002)

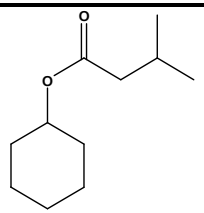
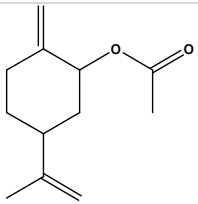
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
02.209 1099	3,3,5-Trimethylcyclohexan-1-ol		3962 116-02-9	Solid $C_9H_{18}O$ 142.24	Insoluble Soluble	193-196 30-34 IR MS 98%	n.a. n.a.	Racemate (EFFA, 2010).
07.033 1115	Isojasmone		3552 167 11050-62-7	Liquid $C_{11}H_{16}O$ 164.24	Insoluble 1 mL in 1mL	144 (13 hPa) NMR 95%	1.472-1.477 0.917-0.924	CAS Nr in Register refers to 2-cyclopenten-1-one, 2-methyl-3-(2-penten-1-yl)-. Additional information indicates that this substance consists of 2-hexyl-2-cyclopent-1-one + 2-pentyl-2-cyclohexen-1-one. However the CAS Nr provided do not correspond with these structures. Therefore, this substance will not be further evaluated in the current revision of this FGE.
07.034 1106	2-Hexylidenecyclopentan-1-one		2573 167 17373-89-6	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Miscible	240 NMR 98%	1.477-1.484 0.907-0.914	Mixture E/Z (50/50) (EFFA, 2012).

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
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		3061 168 17369-60-7	Liquid $C_{12}H_{20}O$ 180.29	Slightly soluble Miscible	113-115 NMR 97%	1.485-1.490 0.927-0.934	Mixture of 5-ethyl-2,3,4,5-tetramethyl-2-cyclohexen-1-one and 5-ethyl-3,4,5,6-tetra-methyl-2-cyclohexen-1-one. The predominant constituent is 5-ethyl-3,4,5,6-tetramethyl-2-cyclohexen-1-one. Mixture of diastereoisomers in approximately equal ratios (EFFA, 2012).
07.045 1108	2,2,6-Trimethylcyclohexanone		3473 686 2408-37-9	Liquid $C_9H_{16}O$ 140.23	Insoluble Miscible	178-179 NMR 99%	1.443-1.449 0.900-0.907	Racemate (EFFA, 2010).
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		3196 11786 488-10-8	Liquid $C_{11}H_{16}O$ 164.25		248 NMR 98%	1.495-1.501 0.942-0.948	According to JECFA: Min. assay value is '98 cis'.
07.095 1109	2-(sec-Butyl)cyclohexanone		3261 11044 14765-30-1	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Miscible	76-78 NMR 94%	1.454-1.461 0.911-0.917	Mixture of diastereoisomers, approximately 25% of each (EFFA, 2012). Min assay 94% secondary comp. 2-isobutyl cyclohexanone 2-2.5% (EFFA, 2010).

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
07.098 1107	3-Methylcyclohex-2-en-1-one		3360 11134 1193-18-6	Liquid $C_7H_{10}O$ 110.16	Miscible Miscible	199-200 NMR 98%	1.490-1.498 0.967-0.972	
07.112 1105	3-Methyl-2-cyclopenten-1-one		3435 11137 2758-18-1	Liquid C_6H_8O 96.12		74 (20 hPa) NMR 98%	1.485-1.491 0.968-0.975	
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		3553 11918 78-59-1	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Miscible	213-215 NMR 97%	1.474-1.481 0.919-0.927	
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		3577 3720-16-9	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Miscible	242-244 NMR 95%	1.481-1.486 0.924-0.928	Racemate (EFFA, 2012)
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		3763 1128-08-1	Liquid $C_{11}H_{18}O$ 166.26	Very slightly soluble Soluble	79 (0.2 hPa) NMR 99%	1.676-1.682 0.911-0.917	
07.148 1100	Cyclohexanone		3909 11047 108-94-1	Liquid $C_6H_{10}O$ 98.14	Miscible	154-156 IR NMR MS 99%	1.447-1.453 0.947-0.950	

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
07.149 1101	Cyclopentanone		3910 11050 120-92-3	Liquid C_5H_8O 84.12	Miscible	130-131 IR NMR MS 99%	1.432-1.438 0.950-0.960	
07.172 1110	4-Isopropylcyclohex-2-en-1-one		3939 11127 500-02-7	Liquid $C_9H_{14}O$ 138.21	Insoluble Miscible	198 NMR 97%	1.481-1.490 0.930-0.950	Racemate (EFFA, 2012).
07.179 1102	2-Methylcyclohexanone		3946 583-60-8	Liquid $C_7H_{12}O$ 112.17	Insoluble Miscible	163-163 IR NMR MS 96%	1.444-1.450 0.924-0.926	Racemate.
07.180 1103	3-Methylcyclohexanone		3947 591-24-2	Liquid $C_7H_{12}O$ 112.17	Insoluble Miscible	169-170 IR NMR MS 97%	1.440-1.450 0.914-0.919	Racemate.
07.257 1117	2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone		3829 68133-79-9	Liquid $C_{15}H_{24}O$ 220.35	Insoluble Miscible	130 (4 hPa) NMR MS 95%	1.482-1.489 0.911-0.916	Racemic mixture of (E)- and (Z)-isomers (EFFA, 2010). The double bond occurs mainly as E-isomer (at least 80% E and max 20% Z) (EFFA, 2012).

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
09.027 1093	Cyclohexyl acetate		2349 217 622-45-7	Liquid $C_8H_{14}O_2$ 142.19	Insoluble Miscible	175-177 NMR 98%	1.436-1.443 0.971-0.978	
09.140 1097	Cyclohexyl propionate		2354 421 6222-35-1	Liquid $C_9H_{16}O_2$ 156.23	Insoluble Miscible	193 NMR 97%	1.439-1.446 0.969-0.974	
09.160 1095	Cyclohexyl formate		2353 498 4351-54-6	Liquid $C_7H_{12}O_2$ 128.17	Insoluble Miscible	162-163 NMR 97%	1.439-1.445 1.052-1.060	
09.230 1094	Cyclohexyl butyrate		2351 2082 1551-44-6	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble Miscible	212 NMR 98%	1.439-1.451 0.953-0.959	

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
09.464 1096	Cyclohexyl isovalerate		2355 459 7774-44-9	Liquid $C_{11}H_{20}O_2$ 184.28	Insoluble Miscible	58-62 NMR 95%	1.439-1.445 0.945-0.952	
09.930 1098	Cyclohexyl, 2-methylene-5-(1-methylethenyl) acetate		3848 71660-03-2	Liquid $C_{12}H_{18}O_2$ 194.27	Insoluble Miscible	77-79 (0.1 hPa) IR NMR MS 95%	1.473-1.479 0.964-0970	Mixtures of diastereoisomers (25% of each) (EFFA, 2012).

(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.

2.4. Genotoxicity data

2.4.1. Genotoxicity studies – Text taken[6] from the JECFA (JECFA, 2003)

Genotoxicity data *in vitro*

Five of the 13[7] alicyclic ketones, secondary alcohols and related esters have been tested for genotoxicity. Overall, negative results were reported in the standard assay for reverse mutation when various strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) were incubated with up to 10,000 µg/plate of cyclohexanone [FL-no: 07.148], 2.5–2500 µg/plate of cyclopentanone [FL-no: 07.149] or up to 4200 µg/plate of 2,2,6-trimethyl cyclohexanone [FL-no: 07.045] with or without metabolic activation (Florin et al., 1980; Haworth et al., 1983). In another test for reverse mutation with *S. typhimurium* TA98, TA100, TA1535 and TA1537 (only an abstract), cyclohexanone was reported to produce 'a large number of revertants' in TA98, with no further elaboration and no results for the other strains. The concentrations and test conditions used were not specified (Massoud et al., 1980).

Both cyclohexyl acetate [FL-no: 09.027] and cyclohexyl butyrate [FL-no: 09.230] gave negative results for mutation in *Bacillus subtilis* M45 (*rec*$^-$) and H17 (*rec*$^+$) (Oda et al., 1979; Yoo, 1986). Positive results were reported with cyclohexanone in an assay for forward mutation assay in *B. subtilis* (Massoud et al., 1980); however, as previously stated, no concentrations or test conditions were reported in the abstract.

Cyclohexanone [FL-no: 07.148] at concentrations up to 980 µg/mL induced chromosomal aberrations in human lymphocytes with or without metabolic activation (Collin, 1971; Lederer et al., 1971; Dyshlovoi et al., 1981). It did not induce chromosomal aberrations in Chinese hamster ovary cells at a concentration of 7.5 µl/mL, with or without metabolic activation (Aaron et al., 1985). In an assay for sister chromatid exchange, cyclohexanone at a concentration of 7.5 µl/mL gave weakly positive results in Chinese hamster ovary cells in the absence of metabolic activation and negative results in the presence of metabolic activation (Aaron et al., 1985).

Genotoxicity data *in vivo*

When cyclohexanone [FL-no: 07.148] was fed to adult *Drosophila melanogaster* for 3 days, no mutations were observed (Goncharova, 1970; Wild et al., 1983; Foureman et al., 1994).

Conclusion

Cyclohexyl acetate, cyclohexyl butyrate, cyclopentanone and 2,2,6-trimethyl cyclohexanone gave negative results in assays for genotoxicity *in vitro*. The results reported for the genotoxicity of cyclohexanone are conflicting. Most of the assays were conducted before 1986, when the pH and ionic strength of test media were often not adequately maintained. Mammalian cells *in situ* rely on complex regulatory mechanisms to maintain homeostatic conditions, and those in culture are not equipped to respond to environmental changes; therefore, it is important that the culture media used in mammalian cell assays be maintained at a pH of approximately 6.8–7.5. A lower pH or changes in osmolality due to the test agents can give rise to false-positive results, especially when metabolic activation systems are added. Acidity facilitates the breakdown of the components of such systems into mutagenic agents (Brusick, 1986).

The equivocal results of the assays for genotoxicity with cyclohexanone *in vitro* can be interpreted in terms of physiochemical properties. Compounds that are structurally similar to cyclohexanone have excellent membrane permeability and hydrogen bonding potential (Slater, 1963; Slater, 1967; Moreland, 1994). When cyclohexanone and related substances are tested *in vitro*, they may induce membrane expansion, leading to multiple effects on membrane-related processes. Membrane expansion may increase cell volume and lipid storage vacuoles, block ionic conductance channels, limit

[6] The text is taken verbatim from the indicated reference source, but text related to substances not included in FGE.51 has been removed.

[7] The genotoxicity data available for the new substances evaluated by JECFA and evaluated by EFSA with respect to genotoxicity concern due to the α, β–unsaturated structures are summarised in Sections 3.3. and 3.4.

the availability of ATP and alter ion fluxes and metabolite distribution between the cytoplasm and organelles. Given these physiochemical properties, it is highly unlikely that any consistent pattern of genotoxicity would result from a battery of assays in bacterial and mammalian cells.

Overall, the tests for genotoxicity yielded mainly negative results. Positive results were reported in mammalian cells at cytotoxic concentrations, usually in the absence of biotransformation enzymes. The *in vivo* assay result was negative.

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

2.4.2. Genotoxicity studies – Text taken[8] from EFSA FGE.09Rev6 (EFSA CEF Panel, 2015b)

Genotoxicity data *in vitro* / *in vivo*

Genoxicity data are available for only three candidate substances cyclohexanol [FL-no: 02.070], cyclopentanol [FL-no: 02.135], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] and for nine supporting substances and one structurally related substances.

Cyclohexanol [FL-no: 02.070] was not genotoxic in two Ames tests and in an *in vivo* micronucleus assay, which are all considered as valid studies. However, the results of the *in vivo* study are of limited relevance, due to the lack of evidence that the substance did reach the bone marrow. Inconclusive results were reported in an *in vitro* chromosomal aberration assay with human leukocytes and negative results were reported in a dominant lethal mutations assay with *Drosophila melanogaster;* both studies were considered inadequate. Cyclopentanol [FL-no: 02.135] was studied in a valid Ames test. No mutagenicity was found.

A battery of *in vitro* and *in vivo* genotoxicity studies were conducted on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] including valid negative reverse mutation tests in *Escherichia coli* (Wagner and Klug, 2000) and *Salmonella typhimurium* (Thompson, 2000).

In a mouse lymphoma test, pre-dating GLP, a more than 2-fold increase of the mutant frequency over the solvent treated control values was found at the highest tested cytotoxic concentration of 300 µg/mL in the presence of metabolic activation, and at the two highest tested cytotoxic concentrations of 200 and 300 µg/mL in the absence of metabolic activation. Only limited documentation is provided in the study report; together with the fact that several cultures were infected and a lack of a confirmatory test, it is impossible to assess the reliability of these results (Ross and Harris, 1979).

No induction of forward mutations at the TK locus in L5178Y mouse lymphoma cells were found in a study performed in compliance with the current OECD test guidelines, both in the absence and in the presence of metabolic activation, up to and including cytotoxic concentrations (Cifone, 2001).

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was tested in a bone marrow micronucleus test in mice following a single intraperitoneal administration of 0, 280, 560 or 1120 mg/kg bw in corn oil. The study was performed in compliance with the current OECD test guidelines. The two highest doses chosen induced clear signs of toxicity; slight reductions (up to 12%) in the ratio of polychromatic erythrocytes to total erythrocytes were found, indicating that the test material had reached the target cells. No increase in micronucleated cells was found in the groups treated with the test material. The positive control induced the expected increases (Gudi and Krsmanovic, 1998).

In an Unscheduled DNA Synthesis (UDS) study, the ability of methyl 3-oxo-2-pentyl-1-cyclopentylacetate to induce DNA repair was studied in isolated rat hepatocytes after administration *in vivo*. The study was performed in compliance with the current OECD Guideline 486 (OECD, 1997). Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was administered to male Sprague-Dawley CD rats by intra-peritoneal injection in doses of 333.3 and 1000 mg/kg bw (the latter dose was the maximum tolerated dose) followed by liver perfusion at 2 or 16 h after dosing. No marked increase in the incidence of UDS was observed at either dose level or perfusion time. Statistically significant differences were revealed in the positive control groups when compared to the negative control group and the test article (Durward, 2001).

[8] The text is taken verbatim from the indicated reference source.

Genotoxicity data are available for nine supporting substances [FL-no: 02.015, 02.062, 07.148, 07.176, 09.027, 09.215, 09.230, 07.149 and 07.045].

Cyclohexanone [FL-no: 07.148], structurally related to the alicyclic ketones and secondary alcohols in this FGE, was not mutagenic in an Ames test, considered to be valid. Negative and positive results were reported in several other *in vitro* studies at gene and chromosomal level, as well as a negative result in a sex-linked recessive lethal mutations in *D. melanogaster*. However, these studies were considered inadequate.

Menthol [FL-no: 02.015] gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes. With the same substance equivocal results in an *in vivo* host mediated mutation assay were observed at high dose levels and negative results in several Ames tests, a TK+/- mouse lymphoma assay, sister chromatid exchange (SCE) tests in Chinese hamster ovary (CHO) cells and human lymphocytes, and chromosomal aberration assays with human embryonic lung cells, human lymphocytes and CHO cells. Negative results were also reported in two *in vivo* micronucleus and chromosomal aberration assays. However, the results of these studies have a limited relevance, due to the lack of bone marrow toxicity. In addition, an *in vivo* dominant lethal assay was available, from which also negative results were obtained. *trans*-Menthone [FL-no: 07.176] was genotoxic in an Ames test and in a somatic mutation and recombination test (SMART) with *Drosophila*. The observed effects were not very pronounced. Further, *trans*-menthone is easily converted to menthol, which is estimated to be overall negative in genotoxicity tests.

Carveol and carvyl acetate [FL-no: 02.062 and 09.215] were tested in Ames test at various doses from 10 - 560 µg/plate in the *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without S9 mix in dimethyl sulphoxide. Positive and negative controls were used. No mutagenicity was observed. (Mortelmans et al., 1986).

Conclusion on genotoxicity

For five of the candidate substances [FL-no: 07.109, 07.202, 07.219, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances.

Only for three of the candidate substances some genotoxicity data are available, and for these three mainly negative results were obtained. For the supporting substances mainly negative, but also some positive results were obtained. The positive results were obtained in poorly reported tests, or in tests, which are difficult to interpret with respect to their relevance for genotoxicity.

Overall, the genotoxic potential of this group of flavouring substances cannot be fully assessed. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA see Tables 4 and 5.

2.4.3. Genotoxicity studies – Text taken[9] from EFSA FGE.211 (EFSA CEF Panel, 2011a)

The following text is relevant for two substances [FL-no: 07.034 and 09.930] in this revision of FGE.51.

The Industry has submitted data concerning genotoxicity studies for the one representative substance for subgroup 2.5, 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 to carvone, which has been evaluated by

[9] The text is taken verbatim from the indicated reference source

EFSA in FGE.212 (EFSA, 2009) and NTP (NTP, 1990a) 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 6 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 table 6).

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 Table 6).

Overall, the Panel concluded that no evidence of chromosomal damage or aneuploidy was observed by increased 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 FGE.211 (subgroup 2.5) would be of no safety concern with respect to genotoxicity.

A summary of the *in vitro* genotoxicity data is given in Table 6.

2.4.4. Genotoxicity studies – Text taken[10] from EFSA FGE.212 (EFSA, 2009), FGE.212Rev1 (EFSA CEF Panel, 2011b) and FGE.212Rev3 (EFSA CEF Panel, 2015a)

The following text is relevant for five substances [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140 and 07.172] in this revision of FGE.51.

[10] The text is taken verbatim from the indicated reference source, but text related to substances not included in FGE.51 has been removed.

For substances evaluated in FGE.212 and FGE.212Rev1

For tetramethyl ethylcyclohexenone (mixture of isomers) [FL-no: 07.035] one *in vitro* and one *in vivo* study are available and 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).

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. 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.

Conclusion on genotoxicity from FGE.212

Isophorone [FL-no: 07.126 (3,5,5-trimethylcyclohex-2-en-1-one)] is genotoxic *in vitro* and since there is some evidence of carcinogenicity in male rats and equivocal evidence of carcinogenicity in male mice and since a non-threshold mechanism could not be excluded based on the data currently available, the Panel concluded that additional data are required for isophorone in order to clarify whether genotoxicity occurs *in vivo* and whether there is a threshold for the effects observed in the target organs in the long-term bioassays. Therefore, an *in vivo* Comet assay in F344/N rats covering these target organs is required in addition to an *in vivo* bone marrow assay with oral application.

Due to structural similarities and lack of data, the remaining substances cannot presently be evaluated through the Procedure [FL-no: 07.035, 07.098, 07.129 and 07.172]. Additional data on genotoxicity are requested for representative substances of this subgroup according to the opinion of the Panel on the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19 (EFSA, 2008c)

Data submitted from Industry in reply to request for additional genotoxicity data in FGE.212

Honma *et al.* (Honma et al., 1999a; Honma et al., 1999b) found that isophorone did not clearly induce mutations in the mouse lymphoma assay (MLA) following 3 h treatments, but observed that it was mutagenic after 24 h 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 8 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 (NTP, 1990b). However, based on Shelby and Witt (Shelby and Witt, 1995), animals should have been sampled at 17 h and, if negative, also at 36 h. The data on the NTP website are only for bone marrow sampled at 36 h. It is therefore possible that a 17 h 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 h in closed metabolic cages. Twenty-four h 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.

In addition, a report by Morishita *et al.* (Morishita et al., 1997) submitted to EPA (EPA, 1997), is relevant and appears to have been previously submitted only as an abstract. This study was designed to investigate whether isophorone and/or α2μ-globulin[11] might be involved in the induction of preputial gland tumours in F-344 rats. 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.
- 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 labelled 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-post labelling assays.

Discussion of the additional data and conclusion

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 in FGE.212Rev1 that there is no concern with respect to genotoxicity of isophorone.

For substances evaluated in FGE.212Rev3

In order to investigate the potential of 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in five strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) in the presence or absence of metabolic activation by S9-mix in two separate experiments. In Experiment 1 the 'plate incorporation assay' was applied, in Experiment 2 treatment with S9-mix included a pre-incubation step (20 min at 37°C). Seven different concentrations of the test substance were tested using appropriate positive control chemicals and purified water as negative control. The highest concentration selected was 5000 μg /plate (range from 5 to 5000 μg/mL and from 80 to 5000 μg/mL in Experiment 1 and 2, respectively). All positive control chemicals

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

induced significant increases in revertant colony numbers, confirming the sensitivity of the tests and the efficacy of the S9-mix, while the negative controls were within the normal ranges. After treatment with 3-methyl-2-cyclopenten-1-one in Experiment 1 evidence of toxicity, in the form of diminished background bacterial lawn, was observed at 5000 µg/mL both in the presence and absence of S9-mix in all strains; in addition, strain TA98 showed toxicity also at 160, 500 and 1600 µg/mL. In most experimental points, at the same concentrations the number of revertant colonies was relatively low. In Experiment 2, toxicity was observed at 5000 µg/mL in all strains but TA102, both in the presence and absence of S9-mix (Bowen, 2014).

No increase in revertant colony numbers was observed at any concentration tested in either the presence and absence of S9-mix. Therefore, it was concluded that 3-methyl-2-cyclopenten-1-one has no mutagenic activity under the conditions employed.

The *in vitro* micronucleus assay was carried out according to the OECD Test Guideline 487 (OECD, 2010) and following GLP. Duplicate cultures of human peripheral blood lymphocytes, prepared from the pooled blood of two female donors and stimulated with phytohaemagglutinin (PHA), were treated with purified water (negative control), 3-methyl-2-cyclopenten-1-one or appropriate positive controls (mitomycin C and noscapine as clastogenic and aneugenic chemicals, respectively, in the absence of S9-mix; cyclophosphamide as a clastogenic chemical in the presence of S9-mix). A single experiment was performed 48 h after mitogen stimulation, following two treatment schedules: 3 + 21 h in the presence and absence of S9-mix, and 24 + 0 h without S9-mix. Micronuclei were analysed at three concentrations (600, 800 and 962 µg/mL; the highest concentration is equivalent to 10mM) chosen on the basis of a preliminary cytotoxicity range-finder Experiment. Applying the 3 + 21 h treatment, the cultures were exposed to 3-methyl-2-cyclopenten-1-one for 3 h either in the presence or the absence of the S9-mix. In the 24 + 0 h treatment cultures were continuously exposed to 3-methyl-2-cyclopenten-1-one for 24 h without the S9-mix. In all cases the cells were harvested 24 h after the beginning of treatment (i.e. 72 h after culture initiation). Four thousand binucleated cells per concentration were analysed. All positive control chemicals induced statistically significant increases in the frequency of micronucleated cells, confirming the sensitivity of the tests and the efficacy of the S9-mix, while the negative controls were within 95^{t}h percentile of the current observed historical vehicle control ranges. At any concentration tested both in the presence and absence of S9-mix, the frequency of binucleated cells with micronuclei was comparable to that of negative controls (values of $p \leq 0.05$ were considered as significant). It was concluded that 3-methyl-2-cyclopenten-1-one did not induce micronuclei in cultured human peripheral blood lymphocytes when tested up to 10 mM under the experimental conditions employed (Watters, 2014).

Conclusion on genotoxicity from FGE.212Rev3

The genotoxicity of the flavouring substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] was assessed by means of two *in vitro* assays (gene mutations in bacteria and micronuclei in human lymphocytes). 3-Methyl-2-cyclopenten-1-one did not induce gene mutations in bacteria with or without metabolic activation when tested under the conditions employed in the study as presented by the applicant. Neither did it induce micronuclei in cultured human blood lymphocytes under the test conditions employed with or without metabolic activation for this study. Therefore, there is no concern with respect to genotoxicity and the substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112] can be evaluated through the Procedure. This conclusion is also valid for three other five-carbon ring substances [FL-no: 07.033, 07.094, 07.140 and 07.219].

Summaries of the *in vitro* and *in vivo* genotoxicity data from FGE.212Rev1 and FGE.212Rev3 are given in Tables 7, 8 and 9.

2.4.5. EFSA considerations on genotoxicity

Data not available for the JECFA at the time of evaluation (59th meeting) for cyclohexanone [FL-no: 07.148] have been considered by EFSA. Results from in vitro genotoxicity studies with cyclohexanone, carried out by NTP, have been published on the NTP website (NTP, 2007). From the technical information also provided there, it can be concluded that the tests by NTP are reliable. A set of Ames tests with Salmonella strains TA98, TA100, TA1535 and TA1537) and a study with mouse lymphoma cells (L5178Y; tk+/-), including cloning efficiency and colony sizing provided convincingly negative results. The tests were carried out with and without metabolic activation at cyclohexanone levels up to

10000 µg/plate in the Ames tests and up to 5000 µg/mL in the mouse lymphoma assay. For a summary of these studies see Table 10.

The Panel noted that cyclohexanone has also been studied in long term carcinogenicity studies in mice (up to 6.2 g/kg bw per day) and rats (up to 0.65 g/kg bw per day) (Lijinsky and Kovatch, 1986). The substance was tested up to the maximum tolerated dose levels and the overall conclusion from these studies was that cyclohexanone is not carcinogenic. In an evaluation of these studies the IARC concluded that the substance was not classifiable as to its carcinogenicity to humans (IARC, 1989).

For eleven candidate substances [FL-no: 07.033, 07.034, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 07.172 and 09.930] it has been concluded in FGE.211, FGE.212Rev1 and FGE.212Rev3, respectively, that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for representative substances. For [FL-no: 07.033], the Panel notes that this evaluation is only applicable to the structures that have been presented for this substance in FGE.212Rev3. However, since it is not clear if these structures actually reflect the chemical substance used as 'isojasmone' the Panel decided that further consideration of [FL-no: 07.033] is not possible.

Therefore, the Panel concluded that the data available do not preclude evaluation of 23 JECFA evaluated alicyclic ketones, secondary alcohols and related esters through the Procedure.

3. Assessment

3.1. Application of the Procedure to 24 alicyclic ketones, secondary alcohols or related esters evaluated by the JECFA (JECFA, 2003)

According to the JECFA six of the substances belong to structural class I and 18 to structural class II using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded all 24 alicyclic ketones, secondary alcohols or related esters at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for structural classes I and II (step A3).

In conclusion, the JECFA evaluated all 24 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 24 substances are summarised in Table 11 (JECFA, 2003).

3.2. Application of the Procedure to 22 secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols by EFSA in FGE.09Rev6 (EFSA CEF Panel, 2015b)

Twenty-two flavouring substances were evaluated in FGE.09Rev6. Fourteen substances are classified into structural class I, seven into structural class II and one into structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

Twenty-one substances were concluded at step A3 using the EFSA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes are below the thresholds of concern for their structural classes (step A3).

For one substance methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] the estimated daily intake exceeds the threshold of concern for structural class II and since the substance is not endogenous the substance proceeds to step A5.

A 90 day study in rats has been performed for [FL-no: 09.520] from which a No Observed Adverse Effect Level (NOAEL) of 100 mg/kg bw per day could be derived. This NOAEL provides a margin of safety of nearly 10^4 compared to the daily intake of 0.013 mg/kg bw per day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Therefore, [FL-no: 09.520] does not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as a flavouring substance.

In conclusion, the Panel considered that 22 of the substances evaluated through the Procedure were of no safety concern at the estimated levels of intakes based on the MSDI approach. For one substance additional data were required.

The stepwise evaluations of the 22 substances are summarised in Table 12 (EFSA CEF Panel, 2015b).

3.3. EFSA considerations

Since for one ([FL-no: 07.033]) of the 24 candidate substances evaluated by JECFA (2003) the chemical identity is not clear ([FL-no: 07.033]), the Panel could only consider the evaluation of 23 JECFA-evaluated substances. The Panel agrees with the application of the Procedure as performed by the JECFA for these 23 substances in the group of alicyclic ketones, secondary alcohols and related esters.

4. Conclusions

The present revision of FGE.51, FGE.51Rev2, is due to new genotoxicity data evaluated in FGE.212Rev3. Based on these data, the Panel concluded that the data available could rule out the concern for genotoxicity for [FL-no: 07.033, 07.094, 07.112 and 07.140] and accordingly these four substances can be evaluated through the Procedure in this revision of FGE.51. Three of these substances were evaluated by the JECFA at its 59th meeting in 2002 [FL-no: 07.033, 07.094 and 07.112] and the fourth was evaluated by the JECFA at its 63rd meeting. These two JECFA evaluations comprise together 57 flavouring substances, five of which are not in the Register and two are no longer supported by industry for use as flavouring substances in Europe. Of the remaining 50 substances, 26 substances have been evaluated in other FGEs or opinions, leaving 24 flavouring substances to be considered in the current revision of FGE.51.

The Panel concluded that the 24 substances in the JECFA flavouring groups of alicyclic ketones, secondary alcohols and related esters and monocyclic and bicyclic secondary alcohols, ketones and related esters are structurally related to the group of secondary alicyclic saturated and unsaturated alcohols, ketones and esters with secondary alicyclic alcohol moieties evaluated by EFSA in Flavouring Group Evaluation 09, Revision 6 (FGE.09Rev6) (EFSA CEF Panel, 2015b).

For one of these 24 substances ([FL-no: 07.033]) the chemical identity could not be unambiguously confirmed. Therefore the Panel could not consider the JECFA evaluation of this substance. Therefore, the current revision of FGE.51 will deal with 23 JECFA-evaluated substances.

For all 23 substances considered in this FGE, the Panel concluded that either they did not raise a concern with respect to genotoxicity, or that concerns with respect to genotoxicity due to the presence of a structural alert for this could be ruled out, based on experimental data.

The Panel agrees with the application of the Procedure as performed by the JECFA for the 23 substances considered in this FGE.

For 23 JECFA evaluated alicyclic ketones, secondary alcohols and related esters [FL-no: 02.209, 07.034, 07.035, 07.045, 07.094, 07.095, 07.098, 07.112, 07.126, 07.129, 07.140, 07.148, 07.149, 07.172, 07.179, 07.180, 07.257, 09.027, 09.140, 09.160, 09.230, 09.464 and 09.930] the Panel agrees with the JECFA conclusion 'no safety concern at estimated levels of intake as flavouring substance' based on the MSDI approach.

In order to determine whether the conclusion for the JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications: Adequate specifications including complete purity criteria and identity tests are available for 21 JECFA-evaluated substances. For [FL-no: 07.094 and 07.112] information on the solubility in water and ethanol is missing and therefore the conclusions on the named substance cannot be applied to the materials of commerce that correspond to these two FL-numbers. For substance [FL-no: 07.033] unambiguous information with respect to the chemical identity should be provided.

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

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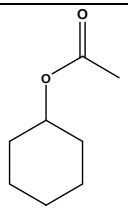
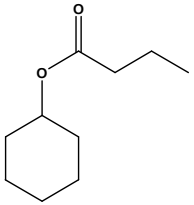
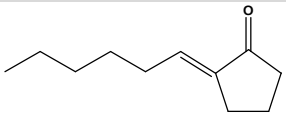
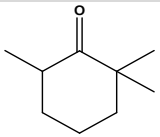
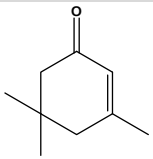
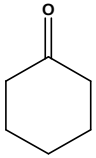
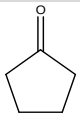
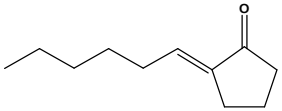
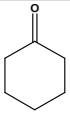
Abbreviations

bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	deoxyribonucleic acid
EPA	United States Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	good laboratory practise
ID	identity
IR	infrared spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MS	mass spectrometry
MSDI	maximised survey-derived daily intake
mTAMDI	modified theoretical added maximum daily intake
NCE	normochromatic erythrocyte
NOEL	no observed effect level
NOAEL	no observed adverse effect level
NMR	nuclear magnetic resonance
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
(Q)SAR	(quantitative) structure-activity relationship
SCE	sister chromatic exchange
SCF	Scientific Committee on Food
WHO	World Health Organization

Appendix A – Summary of genotoxicity and toxicity data

Table 3: Summary of genotoxicity data for alicyclic ketones, secondary alcohols and related esters evaluated by the JECFA (JECFA, 2003)

Chemical Name [FL-no] [JECFA-no]	Structural formula	End-point	Test system	Maximum concentration	Results	Reference
In vitro						
Cyclohexyl acetate 09.027 1093		DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 µg[(d)]/disc	Negative[(a)]	(Yoo, 1986)
Cyclohexyl butyrate 09.230 1094		DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 µg[(d)]/plate	Negative[(a)]	(Oda et al., 1979)
2-hexylidenecyclopentan-1-one 07.034 1106		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 concentrations, up to cytotoxicity or max 3600 µg/plate.	Negative[(a)]	(Wild et al., 1983)
2,2,6-Trimethylcyclohexanone 07.045 1108		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535,TA1537	4.2–3600 µg[(d)]/plate	Negative[(a)]	(Florin et al., 1980)
3,5,5-Trimethylcyclohex-2-en-1-one 07.126		Foreward mutation test	Mouse lymphoma L5178Y Tk$^{+/-}$ cells	0 – 1600 µg/mL	Positive[(b)]	(McGregor et al., 1988)

Chemical Name [FL-no] [JECFA-no]	Structural formula	End-point	Test system	Maximum concentration	Results	Reference
Cyclohexanone 07.148 1100		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–10000 µg[(d)]/plate	Negative[(a)]	(Haworth et al., 1983)
		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.9–2900 µg[(d)]/plate	Negative[(a)]	(Florin et al., 1980)
		Chromosomal	Chinese hamster ovary cells aberration	7.5 µl[(d)]/mL	Negative[(a)]	(Aaron et al., 1985)
		Chromosomal	Human lymphocytes aberration	9.8–980 µg[(d)]/mL	Positive[(a)]	(Lederer et al., 1971)
		Chromosomal	Human lymphocytes aberration	0.005–0.1 µg[(d)]/mL	Positive[(a)]	(Dyshlovoi et al., 1981)
		Sister chromatid exchange	Chinese hamster ovary cells	7.5 µl/mL	Negative[(b)] Positive[(c)]	(Aaron et al., 1985)
Cyclopentanone 07.149 1101		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.5–2500 µg[(d)]/plate	Negative[(a)]	(Florin et al., 1980)
In vivo						
2-hexylidenecyclopentan-1-one 07.034 1106		Sex-linked recessive lethal mutation	*D. melanogaster*	10 mM	Negative	(Wild et al., 1983)
		Micronucleus assay	NMRI mice (4/group)	0, 166, 333, 500 mg/kg bw; single dose, 30 h expression time	Negative	(Wild et al., 1983)
Cyclohexanone 07.148 1100		Sex-linked recessive lethal mutation	*D. melanogaster*	0.1 mL/100 mL	Negative	(Goncharova, 1970)

(a): With and without metabolic activation.
(b): Without metabolic activation.
(c): With metabolic activation.
(d): In the original JECFA report the figures for Maximum concentration were written as 'mg'. This is a mistake by the JECFA as the concentration in the original references is reported in 'µg'. Therefore 'mg' has been replaced by 'µg'.

Table 4: Genotoxicity data (*in vitro*) evaluated by EFSA in FGE.09Rev3 (substances in brackets are JECFA-evaluated substances)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Menthol [02.015])	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	0, and 6 concentrations up to 5000 µg/plate	Negative[(a)]	(Ishidate et al., 1984)	d, l-Menthol was used. The study is considered valid
	Ames test (preincubation method)	*S. typhimurium* TA97, TA98, TA100, TA1535	3–666 µg/plate	Negative[(a)]	(Zeiger et al., 1988)	d, l-Menthol was used. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 5–500 µg/plate	Negative[(a)]	(Nohmi et al., 1985)	d, l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 20–500 µg/plate	Negative[(a)]	(Nohmi et al., 1985)	l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 6.4, 32, 160, and 800 µg/plate	Negative[(a)]	(Andersen and Jensen, 1984)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.
	Ames test	*E. coli WP2 uvrA* (Trp^-)	100–800 µg/plate	Negative	(Yoo, 1986)	l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.
	Ames test	*S. typhimurium* TA97A, TA98, TA100, TA102	0, 5–800 µg/plate	Negative[(a)]	(Gomes-Carneiro et al., 1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.
	Rec assay	*B. subtilis* H17, M45	Up to 10 000 µg/disk	Positive	(Yoo, 1986)	l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method (*rec*-assay) has poor predictive value.
	Rec assay	*B. subtilis* H17, M45	20 µg/disk	Negative	(Oda et al., 1979)	l-Menthol was used. The article is not in English. Only one concentration level is

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (*rec*-assay) has poor predictive value.
	Alkaline elution assay	Rat hepatocytes	0, 0.1–1.3 mM (203.2 µg/mL[(d)])	Negative	(Storer et al., 1996)	The experiment employed *d*-Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.
	Sister chromatid exchange	Chinese hamster ovary cells	5–50 and 0, 2–25 µg/mL[(c)] 0, 16–167 µg/mL[(b)]	Negative[(a)]	(Ivett et al., 1989)	d, l-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/mL[(d)])	Negative[(a)]	(Murthy et al., 1991)	The study is considered valid.
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1, 10 µg/mL	Negative	(Food and Drug Research Laboratories, Inc., 1975)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 and three concentrations up to 200 µg/mL	Negative[(c)]	(Ishidate et al., 1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.
	Chromosome aberration	Chinese hamster ovary cells	0, 50–250 µg/mL	Negative[(a)]	(Ivett et al., 1989)	d, l-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Chromosome aberration	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/mL[(d)])	Negative[(a)]	(Murthy et al., 1991)	The study is considered valid.
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0, 12.5–200 µg/mL	Negative[(a)]	(Myhr and Caspary, 1991)	d, l-Menthol was used. The maximum concentration was selected by a preliminary test The study is considered valid.
(trans-Menthone [07.176])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0, 6.4–800 µg/plate	Positive[(a)]	(Andersen and Jensen, 1984)	Concentrations were selected based on preliminary experiments. In absence of metabolic activation, menthone was mutagenic only to strain TA1537 at 6.4 and 32 µg/mL (slightly less than 2-fold increase in mutation frequency), but not at higher (toxic) concentrations. Also in absence of metabolic

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						activation, there was a concentration dependent increase in number of TA97 strain revertants (up to 4-fold increase at 600 µg/l). It was stated that metabolic activation did not enhance the mutagenicity of menthone. The study is considered valid.
Cyclopentanol [02.135]	Modified Ames test	*S. typhimurium* G46, TA98, TA100, TA1535, C3076, TA1537, D3052, TA1538 *E. coli* WP2, WP2 *uvrA*⁻	0, 0.1–1000 µg/mL	Negative[(a)]	(McMahon et al., 1979)	The study was performed with agar plates containing the following concentration gradients: 0.1 - 1, 1 - 10, 10 - 100, and 100 - 1000 µg/mL. The study is considered valid, although tabulated data on cyclopentanol were not presented.
(Cyclohexanone [07.148])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 33–10 000 µg/plate	Negative[(a)]	(Haworth et al., 1983)	The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 3 µmol/plate	Negative[(a)]	(Florin et al., 1980)	A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03 – 30 µmol/plate. The validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	NR	Positive	(Massoud et al., 1980)	Only an abstract is available. No reporting with respect to metabolic activation. The substance was also tested with *Bacillus subtilis*. With this specie, toxicity was found as well as a positive response. The validity of the study cannot be evaluated because of lack of experimental information.
	Cytogenetic assay	Human leukocytes	0.1–10 mM	Inconclusive[(c)]	(Collin, 1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						studied was not specified. The study is inadequate.
	Chromosomal aberration	Human lymphocytes	0, 0.005–0.1 µg/mL	Positive	(Dyshlovoi et al., 1981)	Article is not in English. Only an abstract available in English. The validity of the study cannot be evaluated.
	Gene mutation (HPRT)	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative[a]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Chromosomal aberration	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative[a]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Sister chromatic exchange	Chinese hamster ovary cells	0, 7.5 µg/ml	Positive[c] Negative[b]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
Cyclohexanol [02.070]	Ames test	*S. typhimurium* TA98, TA1535, TA1537, TA1538	500–10 000 µg/plate[3] 500–15 000 µg/plate[2]	Negative[a]	(Barsky, 1976)	The highest concentrations showed cytotoxicity. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 10–3333 µg/plate	Negative[a]	(Haworth et al., 1983)	The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. Both rat and hamster liver S9 were used. The test was run twice. The study is considered valid.
	Chromosomal aberration	Human leukocytes	0.1–10 mM	Inconclusive[c]	(Collin, 1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.
(Cyclohexyl acetate [09.027])	DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 mg/disc	Negative[a]	(Yoo, 1986)	
(Cyclohexyl butyrate [09.230])	DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 mg/plate	Negative[a]	(Oda et al., 1979)	

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Cycopentanone [07.149])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.5–2500 mg/plate	Negative[(a)]	(Florin et al., 1980)	
(2,2,6-Trimethyl cyclo-hexanone [07.045])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	4.2–3600 mg/plate	Negative[(a)]	(Florin et al., 1980)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535,TA1537	5 mg/plate	Negative[(a)]	(Thompson, 2000)	Valid study in compliance with the OECD Guideline 471.
	Reverse mutation	*E. coli* WP2 *uvrA*	5 mg/plate	Negative[(a)]	(Wagner and Klug, 2000)	Valid study in compliance with the OECD Guideline 471.
	Forward mutation Test	Mouse lymphoma cells *L5178y*	200 & 300µg/L 300 µg/L	Positive[(c)] Positive[(c)]	(Ross and Harris, 1979)	Pre-GLP study - not possible to assess the reliability of these studies.
	Forward mutation Test	Mouse lymphoma cells *L5178y*	100–325 µg/L	Negative[(a)]	(Cifone, 2001)	Valid study and in compliance with OECD Guideline 476.
(Carveol [02.062])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	560 µg/plate	Negative	(Mortelmans et al., 1986)	
(Carvyl acetate [09.215])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	333 µg/plate	Negative	(Mortelmans et al., 1986)	
(L-menthyl (R,S)-3-hydroxybutyrate)	Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative[(a)], [(f)]	(Morimoto, 2005)	The JECFA evaluated the racemate of L-menthyl (R,S)-3-hydroxybutyrate.
	Reverse mutation	E. coli WP2uvrA	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative[(a)], [(f)]	(Morimoto, 2005)	

NA: Not applicable.
NR: Not reported.
(a): With and without S9 metabolic activation.
(b): With S9 activation.
(c): Without S9 activation.
(d): Calculated based on molecular weight of menthol = 156.3 g/mol.
(e): Marked differential toxicity was seen at dose levels above 25 µmol/plate. No observations were noted at lower dose levels.
(f): Modified preincubation method.

Table 5: Genotoxicity data (*in vivo*) evaluated by EFSA in FGE.09Rev3 (substances in brackets are JECFA-evaluated substances)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Reported Result	Reference	Comments
(Menthol [02.015])	Host mediated mutation assay	*S. typhimurium* TA1530 and G46; *S. cerevisiae* D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45–5000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Equivocal	(Food and Drug Research Laboratories, Inc., 1975)	Negative results, with exception of the combination *S. typhimurium* TA1530 – 5000 mg/kg bw and *S. cerevisiae* D3 – 1150 mg/kg bw per day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels.
	In vivo cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45–3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	Oral DL_{50} was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.
	In vivo micronucleus assay	B6C3F1 male mouse bone marrow cells	Intra peritonal	0, 250–1000 mg/kg bw per day, during 3 days	Negative	(Shelby et al., 1993)	d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50% lethality.
	In vivo dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45–3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975)	This study is considered valid.
(trans-Menthone [07.176])	*In vivo* SMART assay	*D. melanogaster* – flr3 × mwh cross	Whole body	0, 1.3 µl/disk	Positive	(Franzios et al., 1997)	Somatic Mutation and Recombination Test. Only one dose level (1.29 µl/disk; slightlyhigher than the LD_{50}) was tested. A two-fold increase in mutation frequency as compared to control was observed. Menthone was not recombinogenic. The validity of this study is unclear.

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Reported Result	Reference	Comments
(Cyclohexanone [07.148])	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/mL	Negative	(Goncharova, 1970)	Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.
Cyclohexanol [02.070]	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/mL	Negative	(Goncharova, 1970)	The validity of the study cannot be evaluated.
	In vivo micronucleus test	NMRI mouse bone marrow	Oral	500–1500 mg/kg bw	Negative	(Gelbke, 1991)	The study is considered valid. The negative result of this study is of limited relevance, since no bone marrow toxicity could be detected. Testing at higher dose levels might not have been possible due to observed general toxicity at the highest dose.
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Micronucleus test	ICR mice	Intra peritonal	280, 560 & 1120 mg/kg bw	Negative	(Gudi and Krsmanovic, 1998)	Valid study in compliance with the OECD Guideline 474.
	Unscheduled DNA Synthesis	Rat hepatocytes	Intra peritonal	333.3 & 1000 mg/kg bw	Negative	(Durward, 2001)	Valid study in compliance with the OECD Guideline 486.

NR: Not reported.

Table 6: Genotoxicity data (*in vitro*) from FGE.211

[FL-no] [JECFA-no]	Chemical Name	Test System	Test Object	Concentrations of substance and test conditions	Result	Reference	Comments
09.930 1098	1(7),8-*p*-Menthadien-2-yl acetate	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA 102	1.6*, 8*, 40*, 200, 1000 and 5000 µg/plate [(a), (b)]	Negative	(Beevers, 2010)	
			S. typhimurium TA98, TA1535 and TA1537	15.6*, 31.3*, 62.5*, 125, 250 and 500 µg/plate [(b), (c)]	Negative		
			S. typhimurium TA100 and TA 102	78.1*, 156.3*, 312.5, 625, 1250 and 2500 µg/plate [(b), (c)]	Negative		
			S. typhimurium TA98 and TA100	156.3*, 312.5, 625, 1250, 2500 and 5000 µg/plate [(d), (e)]	Negative		
			S. typhimurium TA1535, TA1537 and TA 102	78.1*, 156.3*, 312.5, 625, 1250 and 2500 µg/plate [(d), (e)]	Negative		
			S. typhimurium TA100	25*, 50*, 100*, 200 and 400 µg/plate [(b), (c)]	Negative		
			S. typhimurium TA98	50*, 100*, 200*, 400 and 800 µg/plate [(d), (e)]	Negative		
			S. typhimurium TA100, TA1535, TA1537 and TA 102	25*, 50*, 100*, 200 and 400 µg/plate [(d), (e)]	Negative		
		Micronucleus induction	Human peripheral blood lymphocytes	80, 90 and 110 µg/mL [(c), (f)]; 200, 300 and 400 µg/mL [(e), (f)]	Negative	(Whitwell, 2010)	50 to 65% cytotoxicity at top concentrations
				20, 50, 80 and 100 µg/mL [(c), (g)]	Negative		

* concentration without cytotoxicity
(a): With and without S9 metabolic activation.
(b): Plate incorporation method.
(c): Without S9 metabolic activation.
(d): Pre-incubation method.
(e): With S9 metabolic activation.
(f): 3-h incubation with 21-h recovery period.
(g): 24-h incubation with no recovery period.

Table 7: Genotoxicity data (*in vitro*) from FGE.212Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments (e)
Tetramethyl ethylcyclohexenone (mixture of isomers [07.035]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 concentrations up to cytotoxicity, or max. 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Limited validity (no TA 102 or *E. Coli*); possibly slightly low maximal concentration tested.
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	33–10 000 µg/plate	Negative[a]	(Mortelmans et al., 1986)	Valid.
	Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–10 000 µg/plate	Negative[a]	(NTP, 1986)	NTP study carried out according to standard US-EPA guideline; result is considered as valid.
	Mutation	L5178YTk+/– mouse lymphoma cells	67–810 µg/mL	Negative[b]	(McKee et al., 1987)	Validity cannot be evaluated (tested with S9; abstract only with very limited information).
	Mutation	L5178YTk+/– mouse lymphoma cells	130–1300 µg/mL	Negative[c]	(McKee et al., 1987)	Validity cannot be evaluated (tested without S9; abstract only with very limited information).
	Mutation	L5178YTk+/– mouse lymphoma cells	0.089–0.89 µl/mL	Negative[c]	(O'Donoghue et al., 1988)	Valid according to current guidelines.
	Mutation	L5178YTk+/– mouse lymphoma cells	0.13–1.3 µl/mL	Negative[b]	(O'Donoghue et al., 1988)	Valid according to current guidelines.
	Mutation	L5178YTk+/– mouse lymphoma cells	1200 µg/mL	Positive[b]	(NTP, 1986)	NTP study carried out according to standard US-EPA guideline; Not tested with S9. Result is considered as valid.
	Mutation	L5178YTk+/– mouse lymphoma cells	Not reported (however, up to cytotoxic concentrations) for 3 h exposure.	Negative[a]	(Honma et al., 1999a)	Limited validity since data was presented in a summarised table format only (as a result of an international collaborative study).
	Mutation	L5178YTk+/– mouse lymphoma cells	Up to 1500 µg/mL	Positive[b]	(Honma et al., 1999b)	Limited validity since mutation frequencies were not reported in table format. Tested only in the absence of S9. Isophorone was mutagenic after 24 h treatments in the absence of S9. Although only graphs are plotted, it seems that increases in MF that exceeded the Global Evaluation Factor occurred at around 1250-1500 µg/mL where toxicity (by relative survival) reached 70-90%.

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments (e)
	Chromosomal aberration	Chinese hamster ovary cells	5–1600 µg/mL	Negative[(a)]	(Gulati et al., 1989)	Limited validity (not clear if gaps were included in the scores).
	Chromosomal aberration	Chinese hamster ovary cells	250–1600 µg/mL	Negative[(a)]	(NTP, 1986)	NTP study carried out according to standard US-EPA guideline; result is considered as valid.
	Chromosomal aberration	Chinese hamster lung fibroblasts	0–1250[b] µg/mL 0–1500[c] µg/mL	Positive[(a)]	(Matsuoka et al., 1996)	Valid.
	Chromosomal aberration	Chinese hamster lung fibroblasts	250–1000 mg/mL	Negative[(a)]	(Matsuoka et al., 1996)	Valid. Exposed to isophorone without metabolic activation for 24 h or 48 h, cytotoxic at highest concentrations.
	Sister chromatid exchange	Chinese hamster ovary cells	5–1600 mg/mL	Positive[(b), (d)]	(Gulati et al., 1989)	Valid (pos – S9; neg + S9).
	Sister chromatid exchange	Chinese hamster ovary cells	160–1000 mg/mL	Negative[(a)]	(NTP, 1986)	Valid. NTP study carried out according to Standard US-EPA guideline; result is considered as valid.
	Unscheduled DNA synthesis	Rat hepatocytes	0.005–0.4 µl/mL	Negative	(O'Donoghue et al., 1988)	Valid according to current guidelines
	Unscheduled DNA synthesis	Rat hepatocytes	5–200 µl/mL	Negative[(a)]	(McKee et al., 1987)	Validity cannot be evaluated (abstract only with very limited information).
Carvone (isomer not specified)	Gene mutation	*S. typhimurium* TA1535, TA1537, TA98, TA100	3 µmol/plate	Negative	(Florin et al., 1980)	Insufficient validity (spot test, not according to OECD guideline, methods and results insufficiently reported). Isomer (D or L) not reported.
	Rec assay	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	0.6 mL/disc	Negative	(Matsui et al., 1989)	The test system used is considered inappropriate.
d-Carvone [07.146]	Gene mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	333 µg/plate	Negative[(a)]	(NTP, 1990)	Valid
	Gene mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA1537	560 µg/plate	Negative	(Mortelmans et al., 1986)	Valid
	Sister chromatid exchange	Chinese hamster ovary cells	502 µg/mL	Positive[(a)]	(NTP, 1990)	Valid
	Chromosomal aberration	Chinese hamster ovary cells	400 µg/mL	Positive[(a)]	(NTP, 1990)	Valid

(a): With and without S9 metabolic activation.
(b): Without S9 metabolic activation.
(c): With S9 metabolic activation.

(d): Cytotoxic at next highest dose tested (1600 mg/mL)
(e): Validity of genotoxicity studies:
Valid.
Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

Table 8: Genotoxicity data (*in vivo*) from FGE.212Rev1

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments[(a)]
Tetramethyl ethylcyclohexenone (mixture of isomers [07.035]	Sex-linked recessive lethal mutation	*D. melanogaster*	Feed	10 mM	Negative	(Wild et al., 1983)	Limited validity (low nr of chromosomes, limited reporting).
	Micronucleus formation	Mouse bone marrow	i.p.	180, 307, 450 mg/kg bw	Negative	(Wild et al., 1983)	Limited validity. Only analysis at one time point; no PCE/NCE ratio reported.
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Sex-linked recessive lethal mutation	*D. melanogaster*		2000[(b)] and 12 500[(c)] mg/kg	Negative	(Foureman et al., 1994)	Valid, however, only limited relevance.
	Micronucleus formation	CD-1 mice	i.p.	540 mg/kg bw (MTD)	Negative	(McKee et al., 1987)	Validity cannot be evaluated. Abstract only; very limited information no data on PCE/NCE ratio.
	Micronucleus formation	CD-1 mice	i.p.	0.54 mL/kg bw	Negative	(O'Donoghue et al., 1988)	Limited validity. Only one dose level tested, this dose level corresponded to the LD20; sample schedule inadequate.
	Chromosomal aberration	B6C3F1 mice	i.p.	125, 250, 500 mg/kg bw	Negative	(NTP Website, 1990)	Valid. Submitted by Industry in 2009. The standard protocol for in vivo CA is not given on the NTP website. However, based on Shelby and Witt (1995), animals should have been sampled at 17 h and, if negative, also at 36 h. The data on the NTP website are only for bone marrow sampled at 36 h. It is therefore possible that a 17 h sample was also taken, and found to be negative, but the data not 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.
	DNA binding	F344 rats	Gavage	500 mg unlabelled isophorone/kg bw spiked with ^{14}C-isophorone (0.4 mCi/rat)	Negative	(Thier et al., 1990)	Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed.

	DNA binding	B6C3F1 mice	Gavage	500 mg unlabelled isophorone/kg bw spiked with ^{14}C-isophorone (0.08 mCi/mouse)	Negative	(Thier et al., 1990)	Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed.
	DNA binding	F344 rats (10 males)	Gavage	500 mg/kg bw ^{14}C-isophorone (0.1 mCi/rat)	Negative	(Morishita et al., 1997)	Valid. Preputial glands and kidneys were analysed.
	DNA adducts (^{32}P-Postlabelling)	F344 rats (7 males and 7 females per dose group)	Gavage	0 and 500 mg/kg per day for 5 days.	Negative	(Morishita et al., 1997)	Valid. Preputial glands were analysed.

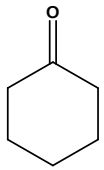
(a): Validity of genotoxicity studies:
Valid.
Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).
(b): Oral administration.
(c): Injection.

Table 9: Genotoxicity data (*in vitro*) from FGE.212Rev3

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments
3-Methyl-2-cyclopenten-2-one [07.112]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA102	5–5000 µg/mL	Negative[(a)]	(Bowen, 2014)	TA98 showed toxicity at 160 µg/mL.
	Micronucleus Assay	Human peripheral blood lymphocytes	600, 800 and 962 µg/mL	Negative	(Watters, 2014)	

(a): With and without S9 metabolic activation.

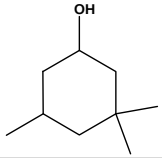
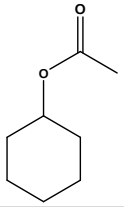
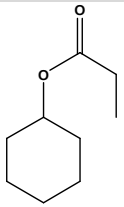
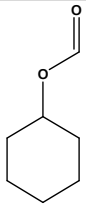
Table 10: Additional genotoxicity data *(in vitro*)

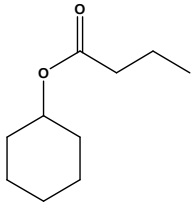
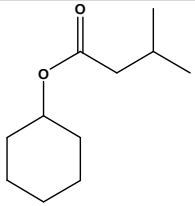
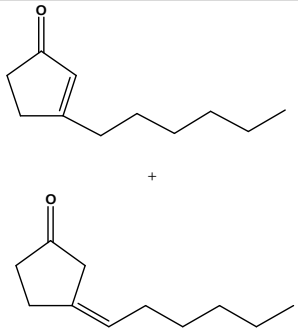
[FL-no]	EU Register name JECFA name	Structural formula	End-point	Test system	Maximum concentration	Results	Reference
07.148 1100	Cyclohexanone		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–3333 µg/plate	Negative[(a)]	(NTP, 2007)
			Mutation	Mouse lymphoma L5178Y $Tk^{+/-}$ cells	312.5–5000 µg/mL	Negative	(NTP, 2007)

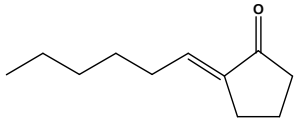
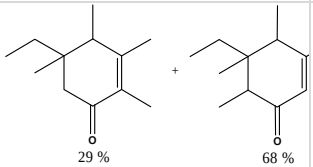
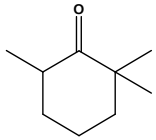
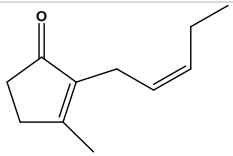
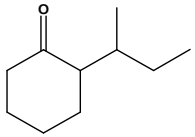
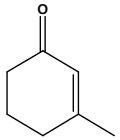
(a): With and without S9 metabolic activation.

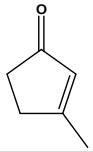
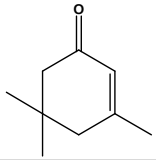
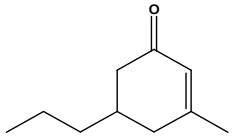
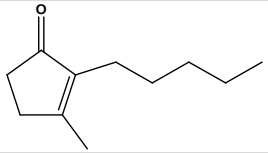
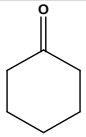
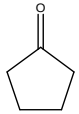
Appendix B – Summary of Safety Evaluations

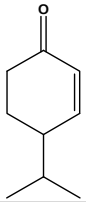
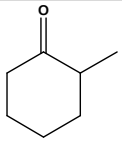
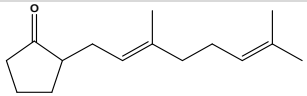
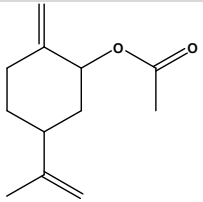
Table 11: Summary of safety evaluation of alicyclic ketones, secondary alcohols and related esters (JECFA, 2003)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI (a) US MSDI (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.209 1099	3,3,5-Trimethylcyclohexan-1-ol		0.12 0.1	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.
09.027 1093	Cyclohexyl acetate		12 10	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.
09.140 1097	Cyclohexyl propionate		0.012 0.05	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.
09.160 1095	Cyclohexyl formate		0.012 0.2	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.

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI (a) US MSDI (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.230 1094	Cyclohexyl butyrate		0.89 0.1	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.
09.464 1096	Cyclohexyl isovalerate		0.28 0.05	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.
07.033 1115	Isojasmone		0.37 0.01	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev3, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	CAS Nr in Register to be checked. CASrn in Register refers to 2-cyclopenten-1-one, 2-methyl-3-(2-penten-1-yl). Additional information indicates that this substance consists of 2-hexyl-2-cyclopent-1-one + 2-pentyl-2-cyclohexen-1-one. However the CAS nrs provided do not correspond with these structures. Therefore, this substance will not be further evaluated in the current revision of this FGE.

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI (a) US MSDI (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.034 1106	2-Hexylidenecyclopentan-1-one		0.24 0.01	Class II 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.	No safety concern at the estimated level of intake based on the MSDI approach.
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		7.8 0.2	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.045 1108	2,2,6-Trimethylcyclohexanone		2.1 0.04	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.
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		13 7.2	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev3, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	According to JECFA: Min. assay value is '98 cis'. No safety concern at the estimated level of intake based on the MSDI approach.
07.095 1109	2-(sec-Butyl)cyclohexanone		5.1 ND	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	According to JECFA: Min. assay value is '94%' and secondary components '2-Isobutyl cyclohexanone' No safety concern at the estimated level of intake based on the MSDI approach.
07.098 1107	3-Methylcyclohex-2-en-1-one		0.012 0.1	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI (a) US MSDI (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.112 1105	3-Methyl-2-cyclopenten-1-one		0.06 ND	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev3, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		4.6 0.1	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		0.097 4.1	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		0.34 0.2	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev3, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.148 1100	Cyclohexanone		0.12 0.1	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.
07.149 1101	Cyclopentanone		0.018 0.02	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.

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI (a) US MSDI (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.172 1110	4-Isopropylcyclohex-2-en-1-one		0.0012 0.001	Class II A3: Intake below threshold	d	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.179 1102	2-Methylcyclohexanone		0.12 0.1	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Racemate. No safety concern at the estimated level of intake based on the MSDI approach.
07.257 1117	2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone		3 6.6	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.
09.930 1098	Cyclohexyl, 2-methylene-5-(1-methylethenyl) acetate		0.61 0.6	Class II 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.	No safety concern at the estimated level of intake based on the MSDI approach.

ND: not determined

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

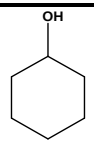
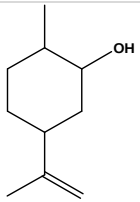
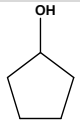
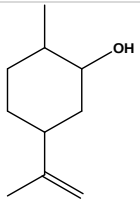
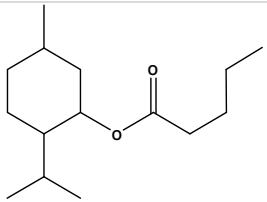
(b): Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.

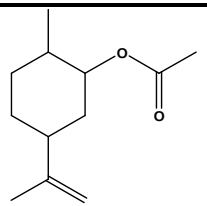
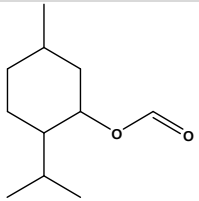
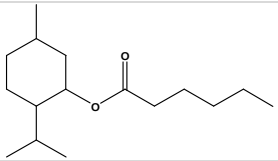
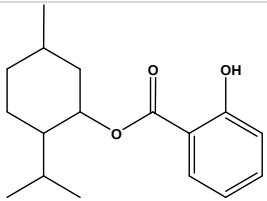
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

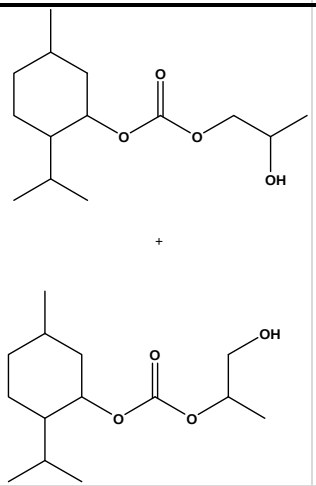
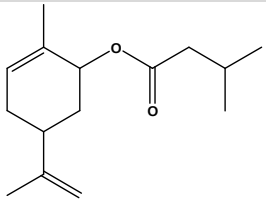
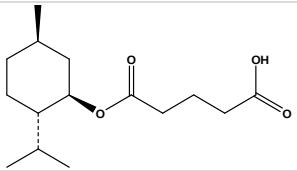
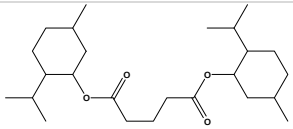
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.

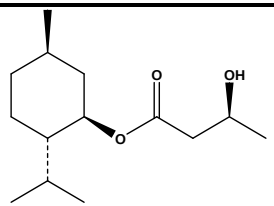
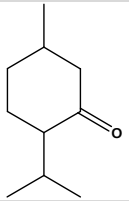
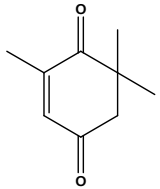
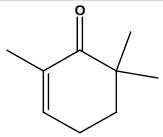
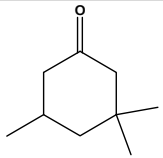
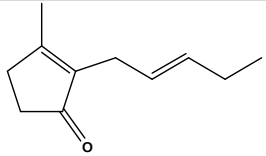
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.

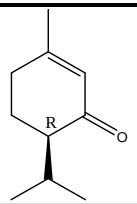
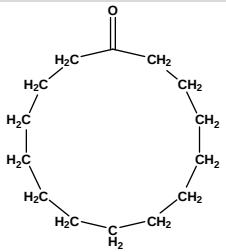
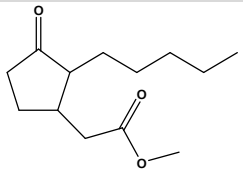
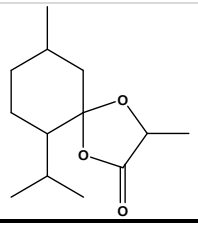
Table 12: Summary of safety evaluation of supporting substances as evaluated in FGE.09rev6 (EFSA CEF Panel, 2015b), applying the Procedure

FL-no JECFA-no	EU Register name	Structural formula	MSDI (a) (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	Outcome on the material of commerce (f), (g), (h)	Evaluation remarks
02.070	Cyclohexanol		3.7	Class I A3: Intake below threshold	d	f	
02.075	(1R,2S,5S)-neo-Dihydrocarveol		2.4	Class I A3: Intake below threshold	d	f	
02.135	Cyclopentanol		0.012	Class I A3: Intake below threshold	d	f	
02.167	(1R,2R,5S)-Isodihydrocarveol		2.4	Class I A3: Intake below threshold	d	f	
09.154 1852	Menthyl valerate		1	Class I A3: Intake below threshold	d	f	

FL-no JECFA-no	EU Register name	Structural formula	MSDI (a) (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	Outcome on the material of commerce (f), (g), (h)	Evaluation remarks
09.355	neo-Dihydrocarvyl acetate		0.012	Class I A3: Intake below threshold	d	f	
09.618	Menthyl formate		0.73	Class I A3: Intake below threshold	d	f	
09.619	(1R,2S,5R)-Menthyl hexanoate		0.37	Class I A3: Intake below threshold	d	f	
09.621	(1R,2S,5R)-Menthyl salicylate		0.012	Class I A3: Intake below threshold	d	f	

FL-no JECFA-no	EU Register name	Structural formula	MSDI (a) (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	Outcome on the material of commerce (f), (g), (h)	Evaluation remarks
09.843	Menthol 1-and 2-propylene glycol carbonate		830 380	Class I A3: Intake below threshold	d	g	
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: Intake below threshold	d	f	Evaluated in FGE.212, genotoxicity concern could be ruled out.
09.929	L-Monomenthyl glutarate		110	Class I A3: Intake below threshold	d	f	
09.935	Dimenthyl glutarate		30	Class I A3: Intake below threshold	d	f	

FL-no JECFA-no	EU Register name	Structural formula	MSDI (a) (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	Outcome on the material of commerce (f), (g), (h)	Evaluation remarks
09.949	L-Menthyl (S)-3-hydroxybutyrate		37	Class I A3: Intake below threshold	d	f	
07.059	p-Menthan-3-one		530 2,500	Class II A3: Intake below threshold	d	f	
07.109 1857	2,6,6-Trimethylcyclohex-2-en-1,4-dione		50	Class II A3: Intake below threshold	d	f	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out.
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		0.12	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev1, genotoxicity concern could be ruled out.
07.203	3,3,5-Trimethylcyclohexan-1-one		0.0085	Class II A3: Intake below threshold	d	f	
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		4.7	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev3, genotoxicity concern could be ruled out.

FL-no JECFA-no	EU Register name	Structural formula	MSDI (a) (µg/capita per day)	Class (b) Evaluation procedure path (c)	Outcome on the named compound (d) or (e)	Outcome on the material of commerce (f), (g), (h)	Evaluation remarks
07.255 1856	l-Piperitone		12	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev1, genotoxicity concern could be ruled out.
07.207	Cyclotetradecanone		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (EFFA, 2009).
09.520 1898	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		770	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	f	
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		1.2	Class III A3: Intake below threshold	d	f	

(a): EU MSDI: Amount added to food as flavour in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.
(b): Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.