EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific Opinion on Flavouring Group Evaluation 72, Revision 1 (FGE.72Rev1): Consideration of aliphatic, branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters evaluated by the JECFA (61st meeting) structurally related to branched- and straight-chain unsaturated carboxylic acids, esters of these and straight-chain aliphatic saturated alcohols evaluated by EFSA in FGE.05Rev2

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 72, Revision 1 (FGE.72Rev1): Consideration of aliphatic, branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters evaluated by the JECFA (61st meeting) structurally related to branched- and straight-chain unsaturated carboxylic acids, esters of these and straight-chain aliphatic saturated alcohols evaluated by EFSA in FGE.05Rev2[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific opinion, published on 5 December 2013, replaces the earlier version published on 16 October 2013*.

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 23 aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters, evaluated by the JECFA at their 61st meeting. This revision is made due to inclusion of one additional substance, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] cleared for genotoxicity concern in FGE.207. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for all 23 substances considered in this FGE and agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. Besides the safety assessment of these flavouring

[1] On request from the European Commission, Question No EFSA-Q-2013-00551, adopted on 25 September 2013.

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

* Minor changes of editorial nature were made. The changes do not affect the contents of this report. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request, as is a version showing all the changes made.

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substances, the specifications for the materials of commerce have also been considered and for all 23 substances, the information is adequate.

KEY WORDS

flavouring safety, JECFA, aliphatic branched-chain, alcohols, aldehydes, acids, FGE.05Rev2

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

In the first version of Flavouring Group Evaluation 72 (FGE.72), EFSA considered a group of 22 aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters which had been evaluated by the JECFA at their 61st meeting.

The present revision of FGE.72 is prepared due to inclusion of one additional substance, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], which has been cleared for genotoxicity concern in FGE.207. Furthermore, European tonnage data for two substances [FL-no: 05.148 and 08.079] as well as information on the stereoisomeric composition for 12 substances [FL-no: 02.011, 02.012, 02.027, 02.029, 05.020, 05.021, 05.148, 08.036, 08.044, 08.055, 08.079 and 09.273] have been provided since the first publication of FGE.72.

The Panel concluded that the 23 substances are structurally related to the group of branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols evaluated by EFSA in the Flavouring Group Evaluation 05, Revision 2 (FGE.05Rev2).

The Panel concluded, based on the genotoxicity data available for substances in FGE.05Rev2 and substances [FL-no: 05.020, 05.124 and 09.931] (FGE.202 and FGE.207), that genotoxicity is not of concern for any of the 23 substances in FGE.72Rev1.

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

For all 23 substances use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 23 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 are available for all 23 JECFA-evaluated substances.

Thus, for all 23 JECFA-evaluated aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters [FL-no: 02.011, 02.012, 02.027, 02.029, 02.058, 02.076, 02.109, 05.020, 05.021, 05.124, 05.148, 05.169, 08.036, 08.044, 08.047, 08.055, 08.064, 08.070, 08.079, 09.273, 09.408, 09.931 and 16.001], the Panel agrees with JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA has evaluated 11 flavouring substances, which correspond to subgroup 1.1.2 of FGE.19, in its evaluation of the flavouring group 201 (FGE.201). The opinion was adopted on 25 September 2008.

EFSA concluded that a genotoxic potential of the 11 α,β-unsaturated aldehydes and alcohol and related esters in the present FGE.201 could not be ruled out.

Information on one representative material 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] has now been submitted by the European Flavour Association. This information is intended to cover also the re-evaluation of the following four substances from FGE.19 subgroup 2.1 (FGE.207):

- 12-beta-Santalen-14-ol [FL-no: 02.216]
- 12-alpha-Santalen-14-ol [FL-no: 02.217]
- Santalyl acetate [FL-no: 09.034]
- Santalyl phenylacetate [FL-no: 09.712]

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

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the following five substances: 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217], santalyl acetate [FL-no: 09.034], santalyl phenylacetate [FL-no: 09.712] and 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] in accordance with Commission Regulation (EC) No 1565/2000.

INTERPRETATION OF THE TERMS OF REFERENCE

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], first allocated to FGE.201, has subsequently been transferred to FGE.207 for evaluation with respect to genotoxicity. Based on the new genotoxicity data submitted, the Panel concluded that [FL-no: 09.931] does not give rise to concern with respect to genotoxicity and can accordingly now be evaluated through the Procedure in FGE.72Rev1.

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

[5] 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.

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

Therefore, the European Commission request EFSA to carry out a safety assessment for 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] in accordance with Commission Regulation (EC) No 1565/2000.

ASSESSMENT

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

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

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

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 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.

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

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

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional

information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 µg per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the 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.

Genotoxicity

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

Specifications

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

Structural Relationship

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

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

The JECFA has evaluated a group of 32 flavouring substances consisting of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters.

In FGE.72, which covered 22 of the 32 JECFA-evaluated substances, the Panel agrees with JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach, for 10 substances [FL-no: 02.058, 02.076, 02.109, 05.124, 05.169, 08.047, 08.064, 08.070, 09.408 and 16.001], and for the remaining 12 substances [FL-no: 02.011, 02.012, 02.027, 02.029, 05.020, 05.021, 05.148, 08.036, 08.044, 08.055, 08.079 and 09.273], the Panel had reservations (no European production volumes available, preventing them to be evaluated using the Procedure, and/or missing data on composition and/or isomerism).

FGE	Opinion adopted	Link	No. of substances
FGE.72	25 November 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1402.htm	22
FGE.72Rev1	25 September 2013		23

The Panel concluded in FGE.207 (EFSA CEF Panel, 2013) that [FL-no: 09.931] does not give rise to concern with respect to genotoxicity and can accordingly now be evaluated through the Procedure in FGE.72Rev1.

The present revision of FGE.72 (FGE.72Rev1) includes also the evaluation of new information which has become available since the publication of the first version of FGE.72. European production volumes have been provided (EFFA, 2010) for the two substances [FL-no: 05.148 and 08.079] and for 12 substances [FL-no: 02.011, 02.012, 02.027, 02.029, 05.020, 05.021, 05.148, 08.036, 08.044, 08.055, 08.079 and 09.273] from previous version, additional information on stereoisomerism has been submitted (EFFA, 2010; EFFA, 2013).

2. Presentation of the Substances in the JECFA Flavouring Group

2.1. Description

2.1.1. JECFA Status

The JECFA has evaluated a group of 32 flavouring substances consisting of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters at the 61st meeting (JECFA, 2004a; JECFA, 2004b).

2.1.2. EFSA Considerations

Fifteen of the 32 JECFA-evaluated substances were directly allocated to FGE.72.

Seventeen of the 32 JECFA evaluated substances are α,β-unsaturated aldehydes and alcohols and related esters. As the α,β-unsaturated structures of these aldehydes and alcohols and related esters are considered to be structural alerts for genotoxicity (EFSA, 2008), these substances have been given special considerations.

Possible genotoxicity of seven of the 17 α,β-unsaturated aldehydes and alcohols and related esters [FL-no: 02.012, 02.029, 02.058, 02.109, 05.020, 05.124 and 05.148] have been considered in FGE.202 (EFSA, 2009b). The Panel concluded that although the substances in FGE.202 have a structural alert for genotoxicity, the data available on one of the substances, citral [FL-no: 05.020], made it possible to conclude that there would be no safety concern with respect to genotoxicity for these substances and that they accordingly could be evaluated through the Procedure in FGE.72.

The genotoxicity of nine of the remaining ten α,β-unsaturated substances [FL-no: 05.033, 05.090, 05.095, 05.105, 05.107, 05.126, 05.178, 09.177 and 09.931] have been evaluated in FGE.201 (EFSA, 2009a). For these substances the Panel concluded that they could not be evaluated through the Procedure on the basis of the data available and concluded that there is a need for additional data before the substances can be re-evaluated. For the substance 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], additional genotoxicity data submitted by the Industry (EFFA, 2012) have been evaluated by the Panel in FGE.207 (EFSA CEF Panel, 2013). Based on these data the Panel concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] does not give rise to concern with respect to genotoxicity and accordingly can be evaluated through the Procedure in FGE.72Rev1. As for the remaining one substance [FL-no: 05.114], which is considered in FGE.200 (EFSA CEF Panel, 2011), a final conclusion as to its genotoxic properties is not yet available. Accordingly, the eight substances [FL-no: 05.033, 05.090, 05.095, 05.105, 05.107, 05.126, 05.178 and 09.177] from FGE.201 and the one substance [FL-no: 05.114] from FGE.200 will not be considered in this revision of FGE.72.

This consideration therefore deals with 23 of the 32 JECFA-evaluated substances.

The Panel concluded that the 23 aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters evaluated by the JECFA are structurally related to the group of branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols evaluated by EFSA in the Flavouring Group Evaluation 05, Revision 2 (FGE.05Rev2) (EFSA CEF Panel, 2010).

2.2. Isomers

2.2.1. Status

The following seven substances [FL-no: 02.011, 02.027, 02.076, 05.021, 08.036, 08.047 and 08.079] in the group of the JECFA evaluated aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters, have a chiral centre and the following 11 substances [FL-no: 02.012, 02.029, 02.058, 05.020, 05.148, 08.044, 08.055, 08.064, 09.273, 09.408 and 09.931] can exist as geometrical isomers.

2.2.2. EFSA Considerations

The information about the stereoisomerism were inadequate for 13 of the substances [FL-no: 02.011, 02.012, 02.027, 02.029, 05.020, 05.021, 05.148, 08.036, 08.044, 08.055, 08.079, 09.273 and 09.931] The Industry has submitted additional information for the 13 substances (EFFA, 2010; EFFA, 2013). The Panel concluded based on these data that the information provided on the stereoisomeric composition is adequate for all the substances.

2.3. Specifications

2.3.1. Status

The JECFA specifications are available for all substances (JECFA, 2003) (See Table 1).

2.3.2. EFSA Considerations

The specifications are considered adequate for all 23 substances.

3. Intake Estimation

3.1. Status

For all 23 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 8.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2003)

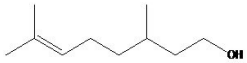
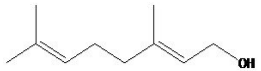
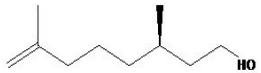
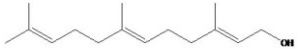
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
02.011 1219	Citronellol		2309 59 106-22-9	Liquid $C_{10}H_{20}O$ 156.27	Slightly soluble Soluble	225 IR 90 %	1.454-1.462 0.850-0.860	Racemate. Min. Assay value 90 %. Other constituents: di-unsaturated and saturated C10 alcohols, citronellyl acetate, citronellal (EFFA, 2010).
02.012 1223	Geraniol		2507 60 106-24-1	Liquid $C_{10}H_{18}O$ 154.25	Slightly soluble Soluble	230 IR 88 %	1.469-1.478 0.870-0.885	The name Geraniol specifies the (*Z*)-isomer (EFFA, 2010). According to JECFA: Min. Assay value is ”88 (total alcohols as C10H18O)” and secondary components ”citronellyl, neryl, and geranyl acetate esters”.
02.027 1222	Rhodinol		2980 76 6812-78-8	Liquid $C_{10}H_{20}O$ 156.27	Insoluble Soluble	132-135 (5 hPa) IR 82 %	1.463-1.473 0.860-0.880	Register name to be changed to (-)-Rhodinol (EFFA, 2010). According to JECFA: Min. assay value is ”82 (total alcohols as C10H20O)” and secondary components ”naturally occurring terpenoid esters - citronellyl, neryl, and geranyl acetate esters”.
02.029 1230	3,7,11-Trimethyldodeca-2,6,10-trien-1-ol		2478 78 4602-84-0	Liquid $C_{15}H_{26}O$ 222.37	Insoluble Soluble	263 IR 96 %	1.487-1.492 0.884-0.889	Mixture of (*Z*)- and (*E*)-isomers for both C=C double bonds (EFFA, 2010). 10-15 %(2*Z*,6*Z*); 20-25 % (2*E*,6*Z*); 20-25 % (2*Z*,6*E*); 40-50 %

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2003)

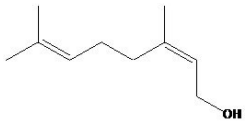
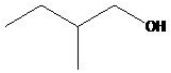
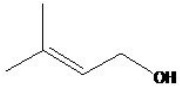
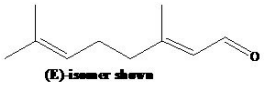
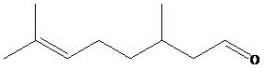
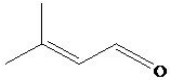
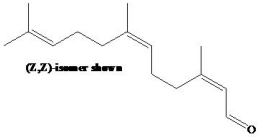
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
								(2*E*,2*E*) (EFFA, 2013).
02.058 1224	Nerol		2770 2018 106-25-2	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Soluble	227 IR 95 %	1.467-1.478 0.875-0.880	Register name to be changed to (*Z*)-Nerol. According to JECFA: Min. assay value is ”95 % (of total alcohols as $C_{10}H_{18}O$)”.
02.076 1199	2-Methylbutan-1-ol		3998 2346 137-32-6	Liquid $C_5H_{12}O$ 88.15	Very slightly soluble Soluble	130 IR NMR MS 99 %	1.409-1.412 0.815-0.820	Racemate.
02.109 1200	3-Methylbut-2-en-1-ol		3647 11795 556-82-1	Liquid $C_5H_{10}O$ 86.10	Insoluble Soluble	140 IR NMR MS 99 %	1.438-1.448 0.844-0.852	
05.020 1225	Citral		2303 109 5392-40-5	Liquid $C_{10}H_{16}O$ 152.24	Very slightly soluble Soluble	228 IR 96 %	1.486-1.490 0.885-0.891	Mixture of (*Z*)- and (*E*)-isomer (EFFA, 2010). CASrn in Register does not specify stereoisomeric composition.
05.021 1220	Citronellal		2307 110 106-23-0	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Soluble	206 IR 85 %	1.446-1.456 0.850-0.860	Racemate. Secondary components: 1,8-cineole, 2-isopropylidene-5-methylcyclohexanol, linalool and citronellyl acetate (EFFA, 2010).
05.124 1202	3-Methylcrotonaldehyde		3646 10354 107-86-8	Liquid C_5H_8O 84.11	Slightly soluble Soluble	133-135 IR NMR 99 %	1.458-1.464 0.870-0.875	
05.148 1228	Farnesal		4019 19317-11-4	Liquid $C_{15}H_{24}O$ 220.36	Insoluble Soluble	198-201 (10hPa) IR NMR MS 99 %	1.494-1.504 0.890-0.900	Mixture of (*Z*)- and (*E*)-isomer for both C=C double bonds (EFFA, 2010). 10-15 % (2Z,6Z); 20-25 % (2E,6Z); 20-25

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2003)

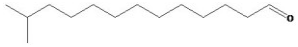
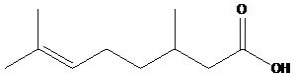
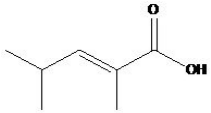
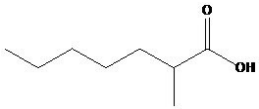
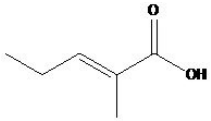
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
								% (2*Z*,6*E*); 40-50 % (2*E*,2*E*) (EFFA, 2013).
05.169 1229	12-Methyltridecanal		4005 75853-49-5	Liquid $C_{14}H_{28}O$ 212.38	Insoluble Soluble	141-143 (5 hPa) IR NMR MS 97 %	1.445-1.455 0.930-0.941	
08.036 1221	Citronellic acid		3142 616 502-47-6	Liquid $C_{10}H_{18}O_2$ 170.25	Insoluble Soluble	121-122 (1 hPa) NMR 90 %	1.455-1.462 0.920-0.926 (20°)	Racemate. Min. assay value (90 %). Other main constituents: citronellal; citronellyl acetate, nerol and geraniol (EFFA, 2010).
08.044 1211	2,4-Dimethylpent-2-enoic acid		3143 744 21016-46-6	Liquid $C_7H_{12}O_2$ 128.17	Very slightly soluble Soluble	133-134 (20hPa) NMR 92 %	1.459-1.467 0.991-0.999	(*E*)-isomer (92 %), other const. 4-methyl-2-methylenevaleric acid (EFFA, 2010). Register name to be changed to (2E),4-Dimethylpent-2-enoic acid. According to JECFA: Min. Assay value ”92 (sum of isomers)” and secondary components ”4-methyl-2-methylenevaleric acid”.
08.047 1212	2-Methylheptanoic acid		2706 2003 1188-02-9	Liquid $C_8H_{16}O_2$ 144.21	Very slightly soluble Soluble	121-122 (17hPa) NMR 97 %	1.420-1.427 0.899-0.905	Racemate.
08.055 1210	2-Methyl-2-pentenoic acid		3195 11680 3142-72-1	Liquid $C_6H_{10}O_2$ 114.14	Slightly soluble Soluble	123-125 (39hPa) IR 98 %	1.450-1.460 0.976-0.982	Mixture of (*Z*)- and (*E*)-isomer (EFFA, 2010). CASrn in Register does not specify stereoisomeric composition. 60-75 % (*E*) and 20-30 % (*Z*) (EFFA, 2013).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2003)

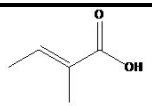
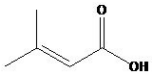
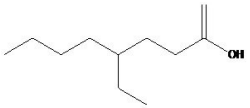
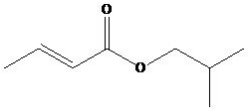
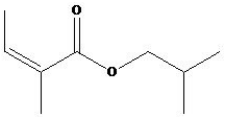
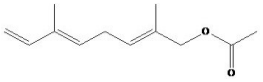
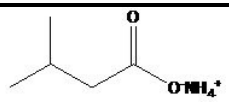
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
08.064 1205	2-Methylcrotonic acid		3599 10168 80-59-1	Solid $C_5H_8O_2$ 100.10	Slightly soluble Soluble	n.a. 61-67 MS 99 %	n.a. n.a.	Register name to be changed to (2*E*)-Methylcrotonic acid.
08.070 1204	3-Methylcrotonic acid		3187 10138 541-47-9	Solid $C_5H_8O_2$ 100.12	Soluble Soluble	 70 MS 98 %	n.a. n.a.	
08.079 1218	4-Ethyloctanoic acid		3800 16493-80-4	Liquid $C_{10}H_{20}O_2$ 172.27	Slightly soluble Soluble	110 (1 hPa) IR NMR 99 %	1.430-1.439 0.898-0.908	Racemate (EFFA, 2010).
09.273 1206	Isobutyl crotonate		3432 10706 589-66-2	Liquid $C_8H_{14}O_2$ 142.20	Slightly soluble Soluble	171 IR 95 %	1.426-1.430 0.880-0.900	Mixture of (*Z*)- and (*E*)-isomer (EFFA, 2010). CASrn in Register does not specify stereoisomeric composition. 70-85 % (*E*) and 10-35 % (*Z*) (EFFA, 2013).
09.408 1213	Isobutyl 2-methylbut-2(cis)-enoate		2180 247 7779-81-9	Liquid $C_9H_{16}O_2$ 156.23	Insoluble Soluble	176-177 IR NMR 98 %	1.438-1.446 0.874-0.880	
09.931 1226	2,6-Dimethyl-2,5,7-octatriene-1-ol acetate		3886 999999-91-4	Liquid $C_{12}H_{18}O_2$ 194.28	Insoluble Soluble	70 (3 hPa) MS 95 %	1.490-1.500 0.937-0.947	According to JECFA: Min. assay value is 96 % (sum of isomers). 14-20 % (2*Z*,5*Z*); 33-40 % (2*Z*,5*E*); 14-19 % (2*E*,5*Z*); 26-33 % (2*E*,5*E*) (EFFA, 2013). CASrn to be changed to: 197098-61-6.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2003)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
16.001 1203	Ammonium isovalerate		2054 464 7563-33-9	Solid $C_5H_{13}O_2N$ 119.16	Soluble Soluble	n.a. 72 NMR 98 %	n.a. n.a.	

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.

4. Genotoxicity Data

4.1. Genotoxicity Studies – Text Taken[7] from the JECFA Report (JECFA, 2004b)

In vitro

No evidence of mutagenicity was reported in standard or modified (pre-incubation method) Ames assays when *dl*-citronellol [FL-no: 02.011] (up to 85 µg/plate), citronellal [FL-no: 05.021] (up to 500 µg/plate), geraniol [FL-no: 02.012] (up to 889 µg/plate), citral [FL-no: 05.020] (up to 700 µl/plate) and farnesol [FL-no: 02.029] (Register name 3,7,11-trimethyldodeca-2,6,10-trien-1-ol) (up to 5000 µl/plate) were incubated with *Salmonella typhimurium* strains TA92, TA94, TA97a, TA98, TA100, TA102, TA1535, and/or TA1537 with and without metabolic activation (Rockwell and Raw, 1979; Eder et al., 1980; Florin et al., 1980; Kasamaki et al., 1982; Lutz et al., 1982; Ishidate et al., 1984; Zeiger et al., 1987; Creutziger, 1989; Gomes-Carneiro et al., 1998; NTP, 2003). Negative results were reported in a mutation test in which 100 µg/plate of citral was incubated with *Escherichia coli* WP2 *uvr*A (Yoo, 1986).

Citronellal [FL-no: 05.021] and geraniol [FL-no: 02.012] did not induce sister chromatid exchanges in Chinese hamster ovary cells in the absence of metabolic activation at concentrations up to 100 µmol/l (15.4 µg/ml) for citronellal and 333 µmol/l (51.4 µg/ml) for geraniol (Sasaki et al., 1989). In a non-standard assay designed to maximise the frequency of chromosomal aberrations in a Chinese hamster B241 cell line, citronellal at concentrations of 0.008 µg/ml gave weakly positive results with and without metabolic activation (Kasamaki et al., 1982). No evidence of an increase in chromosomal aberrations was reported when geraniol [FL-no: 02.012] at concentrations of up to 125 µg/ml was incubated with Chinese hamster fibroblast cells in the absence of metabolic activation (Ishidate et al., 1984), although there was an 8 % increase in polyploidy.

Assays for sister chromatid exchanges with citral [FL-no: 05.020] were performed in Chinese hamster ovary cells. In the absence of metabolic activation, an increase in sister chromatid exchanges of at least 20 % that of control cultures was reported at concentrations of 0.289 - 2.89 µg/ml in the first trial and 7.5 - 10 µg/ml in the second trial. Toxicity was observed at 8.86 and 20 µg/ml in the first and second trial, respectively. With metabolic activation, an increase of sister chromatid exchanges of at least 20 % that of control cultures was reported with citral at 8.68 µg/ml in the first trial and 15.1 - 40.2 µg/ml in the second trial. Toxicity was reported at 28.9 µg/ml in the first trial; no toxicity was observed in the second trial. Owing to cell cycle delay induced by citral, at the higher concentrations (10 µg/ml without and 20.1 - 40.2 µg/ml with metabolic activation) extended culture periods were necessary to allow accumulation of sufficient second-division metaphase cells for analysis (NTP, 2003). In contrast to these findings, there was no evidence for an increase in chromosomal aberrations with higher concentrations of citral (12.5 - 25.3 µg/ml without and 30.3 - 60.6 µg/ml with metabolic activation) (NTP, 2003) or, in another chromosomal aberration assay in Chinese hamster fibroblast cells, at concentrations of citral of up to 30 µg/ml, without metabolic activation (Ishidate et al., 1984).

Rec assays for DNA repair in *Bacillus subtilis* strains M45 and H17 have been performed with *dl*-citronellol [FL-no: 02.011], citronellal [FL-no: 05.021], geraniol [FL-no: 02.012] and citral [FL-no: 05.020]. In one study, each of the four agents gave negative results at concentrations of 16 or 17 µg/disc (Oda et al., 1979). Citral gave positive results in two other rec assays (Kuroda et al., 1984; Yoo, 1986) but only at high concentrations (1110 and 2220 µg/disc). Rec assays performed at lower concentrations of citral (up to 560 µg/disc) were negative (Kuroda et al., 1984).

In a recently developed assay for DNA damage measuring induction of p53 tumour suppressor protein in mouse fibroblasts (NTCT 929 cell line) *in vitro*, citral gave positive results at concentrations of 10 - 30 µg/ml after 17 hours of incubation. In this assay, increased expression of p53 is considered to indicate the induction of DNA damage (Duerksen-Hughes et al., 1999).

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

In vivo

Groups of four or five male $B63CF_1$ mice received citral [FL-no: 05.020] at a dose of 250, 500, 750 and 1000 mg/kg bw per day by intraperitoneal injection at 24-hour intervals for a period of 3 days. A group of animals given corn oil only and another group given cyclophosphamide were used as vehicle and positive controls, respectively. The highest dose of citral was lethal and only the three lower doses were used to evaluate the results of the assay. Twenty-four hours after the third injection, the animals were sacrificed and blood smears were taken from bone marrow cells collected from the femur. Scoring of 2000 polychromatic erythrocytes for formation of micronuclei revealed no increase in micronucleated polychromatic erythrocytes at any dose. The ratio of polychromatic erythrocytes (PCE) / normochromatic erythrocytes (NCE) was not determined (NTP, 2003).

In addition to the assay for micronuclei formation in bone marrow, an assay for micronuclei formation in mouse peripheral blood erythrocytes was performed. Peripheral blood samples were obtained within 24 hours of the final treatment in a 14-week study of toxicity in which female and male $B63CF_1$ mice were given diet containing microencapsulated citral at a dose of up to 7550 and 8110 mg/kg bw per day, respectively. Blood smears were made, fixed and stained and 1000 NCEs per animal were scored for the frequency of micronuclei. In addition, the percentage of PCEs among the total population of erythrocytes was scored. Results for all doses in both males and females showed no increase in micronucleated NCEs or in the percentage of PCEs (NTP, 2003).

Conclusion on genotoxicity

Several aliphatic branched-chain unsaturated alcohols and aldehydes have been tested in the Ames assay and found to be not mutagenic *in vitro*. In addition to showing a lack of mutagenic potential in the Ames assay, citral gave negative results in assays for mutagenicity in *E. coli* WP2 *uvrA*. There was some evidence of DNA damage caused by citral from two rec assays with *B. subtilis*, but only at very high concentrations. Rec assays performed with lower concentrations of test substance, however, gave negative results for citral as well as for *dl*-citronellol, citronellal and geraniol.

Citronellal showed weak evidence of clastogenicity in a non-standard assay for chromosomal aberrations, but gave negative results in assays for sister chromatid exchanges. Geraniol neither induced sister chromatid exchanges nor chromosomal aberrations. Citral showed evidence of activity in assays for sister chromatid exchanges, but increased incubation times were required because of delayed cell cycling. Citral did not induce chromosomal aberrations *in vitro* nor did it show signs of genotoxicity in assays for micronucleus formation in bone marrow and peripheral erythrocytes *in vivo*. Citral induced DNA damage in mouse fibroblasts *in vitro*, as shown by increased expression of P53. This result, however, contrasts with the results of existing assays for genotoxicity with citral, which are largely negative.

On the basis of the results of available studies of genotoxicity, the Committee concluded that members of this group of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters are not genotoxic.

For a summary of *in vitro / in vivo* genotoxicity data considered by the JECFA, see Table 2.

4.2. Genotoxicity Studies – Text Taken[8] from EFSA FGE.05Rev2 (EFSA CEF Panel, 2010)

There are *in vitro* genotoxicity data for four candidate substances [FL-no: 09.375, 09.586, 09.647 and 09.652] and for four supporting substances [FL-no: 08.013, 05.074 and a mixture of 09.646 and methyl linolenate]. *In vivo* data are available for two candidate substances [FL-no: 09.586 and 09.647] and for one supporting substance [FL-no: 05.074].

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

Studies on candidate substances

In vitro studies

Methyl oleate [FL-no: 09.652], methyl methacrylate [FL-no: 09.647], ethyl methacrylate [FL-no: 09.375] and isobutyl 2-methylprop-2-enoate [FL-no: 09.586] were reported to be non-mutagenic in standard, pre-incubation or liquid suspension protocol Ames assays including *S. typhimurium* strains TA97, TA98, TA100, TA1535, TA1537 and/or TA1538 with or without metabolic activation (Table 3). In three instances, the results of Ames assays with methyl methacrylate were weakly positive; however, these results were accompanied by cytotoxicity.

Methyl methacrylate and ethyl methacrylate have been tested in several mammalian cell assays (Table 3). Positive results seen in chromosome aberrations, mouse lymphoma, Sister Chromatid Exchange (SCE), Hypoxanthine Phosphoribosyl Transferase (HPRT) and/or micronucleus assays in most instances were obtained at high exposure concentrations (i.e. > 10 mM or > 1000 µg/ml) and (when reported) high levels of cytotoxicity. However, when methyl methacrylate was tested in a mouse lymphoma assay at concentrations between 5 and 10 mM in the presence of S9-mix, it revealed a positive result which was accompanied by only low cytotoxicity (about 80 % survival at 5 mM and approximately 40 % at 10 mM) (Dearfield et al., 1991).

In vivo studies

Methyl methacrylate [FL-no: 09.647] was evaluated in a mouse micronucleus study conducted by oral gavage. The result was negative, however, it is not clear whether the substance had reached the bone marrow. Two sex-linked recessive lethal mutation studies (one by inhalation and the other by injection) in *Drosophila melanogaster* were negative, as was a dominant lethal assay in mice conducted via inhalation exposure. Rats exposed to high inhalation concentrations of methyl methacrylate did have weak, but statistically significant, increases in chromosome aberrations in bone marrow cells at some exposure levels in comparison to the negative control values both after single and multiple exposures. However, a clear conclusion cannot be drawn from these studies. SCE and chromosome aberration studies with peripheral lymphocytes from male workers occupationally exposed to methyl methacrylate by inhalation for eight hours/day were negative (Table 4).

Isobutyl 2-methylprop-2-enoate [FL-no: 09.586] was evaluated in a mouse micronucleus study with oral doses as high as 5000 mg/kg bw. Results were reported to be negative.

For methyl methacrylate, genotoxicity data were summarised the EU Risk Assessment Report (CEC, 2002) as follows:

"Methyl methacrylate was negative in bacterial gene mutation tests. From mammalian cell culture assays it may be concluded that methyl methacrylate is a high toxicity clastogen (i.e. induction of chromosomal aberrations is bound to highly toxic doses). The effect is not dependent on presence of S9-mix. These findings are in line with results from mouse lymphoma assays where positive findings seem to be due to the induction of small colonies. Marginal increases in SCE frequencies are of low significance."

"*In vivo* an oral mouse bone marrow micronucleus test was negative for doses up to 4520 mg/kg. No clear conclusion could be drawn from bone marrow chromosomal aberration assays with rats. A dominant lethal assay with male mice led to a negative result."

"*In vitro* methyl methacrylate has the potential for induction of mutagenic effects, esp. clastogenicity; however, this potential seems to be limited to high doses with strong toxic effects. Furthermore, the

negative *in vivo* micronucleus test and the negative dominant lethal assay indicate that this potential is probably not expressed *in vivo*."

Studies on supporting substances

In vitro studies

No evidence of mutagenicity was reported for 2,6-dimethyl-5-heptenal [FL-no: 05.074], oleic acid [FL-no: 08.013], methyl linolenate [FL-no: 09.646] or methyl linoleate [FL-no: 09.645] in the standard or pre-incubation protocol Ames assay using *S. typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 with or without the addition of metabolic activation (Shimizu et al., 1985; Mortelmans et al., 1986; Wild et al., 1983; Heck et al., 1989) (Table 3). The maximum doses reported for these studies ranged from 333 to 50000 µg/plate. In further bacterial assays, such as the rec-assay utilising *B. subtilis*, incubated with oleic acid (Osawa and Namiki, 1982), the His^+ reversion assay utilising *S. typhimurium* incubated with methyl linoleate or methyl linolenate [FL-no: 09.646] (MacGregor et al., 1985) and a modified Ames test utilising *E. coli* WP*2uvrA* incubated with oleic acid (Shimizu et al., 1985), these aliphatic unsaturated non-conjugated acids and esters were non-mutagenic.

With respect to mammalian cell assays, rat hepatocytes were tested for unscheduled DNA synthesis (UDS) after exposure to concentrations of up to 1.0 mg 2,6-dimethyl-5-heptenal/ml [FL-no: 05.074] (Table 3). The results from this study showed no genotoxic effects (Heck et al., 1989).

In vivo studies

A bone marrow micronucleus test was conducted *in vivo* in mice with a maximum single dose of 1540 mg/kg 2,6-dimethyl-5-heptenal [FL-no: 05.074]. All mice survived the treatment. There were no statistically significant increases in the incidence of micronucleated PCEs observed (Wild et al., 1983). However, the quality of the study is limited since only a single sampling time was used and the PCE/NCE ratio was not reported. Therefore, it is not clear whether the substance had reached the bone marrow.

In the *Basc* test using *D. melanogaster*, 2,6-dimethyl-5-heptenal was negative when tested at a concentration of 25 mM (Wild et al., 1983).

Conclusion on genotoxicity

Genotoxicity data are available only for a limited number of substances and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substances using the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data considered by the EFSA in FGE.05Rev2, see Tables 3 and 4.

4.3. Genotoxicity Studies – Text Taken[9] from EFSA FGE.202 (EFSA, 2009b)

"There are *in vitro* and *in vivo* studies available on citral [FL-no: 05.020] and on 3-methylcrotonaldehyde (3-methyl-2-butenal) [FL-no: 05.124].

3-Methylcrotonaldehyde was found mutagenic in a valid modified Ames test, i.e. the liquid suspension assay, both in the absence, and to a lower extent, in the presence of metabolic activation (S9-mix), in TA100 *S. typhimurium* strain (BASF, 1991). Of doubtful relevance was a slight increase (factor 2.1) in the number of revertants observed with TA98 strain, only in the absence of S9-mix at the highest

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

concentration (2500 µg/plate). It was found negative in a valid bone marrow micronucleus assay in mice, treated orally at 175, 350 and 750 mg/kg body weight, with signs of toxicity at the highest dose, as shown by the ratio of PCEs/NCEs (BASF, 1992). Moreover, it was found negative in a valid *in vivo* unscheduled DNA synthesis (UDS) assay, carried out on hepatocytes from rats treated orally at dose levels of 350 and 700 mg/kg body weight (BASF, 2001). In conclusion, based on the negative results in two valid *in vivo* assays (rat liver UDS and mouse bone marrow micronucleus), the positive result observed in the modified Ames test is considered of limited relevance for the overall evaluation. Therefore, for this substance, the Panel considers that genotoxicity is of no concern.

Citral was not mutagenic in several valid Ames tests (Gomes-Carneiro et al., 1998; NTP, 2003; Ishidate et al., 1984; Zeiger et al., 1987) and it did not induce chromosome aberrations in a valid *in vitro* study with chinese hamster ovary (CHO) cells (NTP, 2003). Moreover, it was negative in a valid *in vivo* mouse bone marrow micronucleus assay (NTP, 2003). The positive results in an *in vitro* test for sister chromatid exchanges (SCE) (NTP, 2003) and in inappropriate test systems like the Rec assay in *B. subtilis* (Yoo, 1986; Kuroda et al., 1984) and the induction of the tumour suppressor protein p53 (Duerksen-Hughes et al., 1999) are considered of limited relevance for the overall evaluation. The Panel concluded that for citral genotoxicity is not of concern.

Overall, the Panel concluded that the genotoxicity data available do not give rise to concern for the 37 substances in FGE.202 using the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data considered by the EFSA in FGE.202, see Tables 5 and 6

Conclusion on Genotoxicity and Carcinogenicity

Based on the available data, the Panel concluded that there would be no safety concern with respect to genotoxicity or carcinogenicity for the 37 α,β-unsaturated substances presented in this FGE."

4.4. Genotoxicity Studies – Text Taken[10] from EFSA FGE.207 (EFSA CEF Panel, 2013)

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

***In vitro* Data**

Bacterial Reverse Mutation Assay

An Ames assay was conducted in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 to assess the mutagenicity of 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], both in the absence and in the presence of metabolic activation by S9-mix (from livers of rats induced with Aroclor 1254), in three experiments (King, 2000). An initial experiment was carried out in the absence and presence of S9-mix in the five strains, using final concentrations of 2,6-dimethyl-2,5,7-octatriene-1-ol acetate at 5 - 5000 µg/plate in the presence of S9-mix activation and 5 - 1500 µg/plate in the absence of S9-mix, plus negative (solvent) and positive controls. The standard plate incorporation assay was used. Evidence of toxicity, in terms of a decrease in revertant count, was apparent on all plates treated at 500 µg/plate and above in the absence of S9-mix. In the presence of S9-mix, the test article was toxic at concentrations of 1500 µg/plate and above for strains TA1537 and TA102, and at 5000 µg/plate for strains TA98, TA100 and TA1535. In all cases revertant counts were obtained from at least four different concentrations, so these data were considered valid for mutation assessment. In the absence of S9-mix activation, no statistically significant increases in revertant numbers were observed in any of the test strains. In the presence of S9-mix activation no statistically significant increases in revertant numbers were observed for strains TA98, TA100, TA1535 or TA1537, but very

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

small increases in revertant numbers were observed in strain TA102 at 15 and 50 μg/plate which, although statistically significant ($p \leq 0.05$), amounted to only 1.17-fold and 1.18-fold increases over background, respectively. Furthermore, no increases were observed at the higher test concentrations of 150 and 500 μg/plate.

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

On this basis, the very small increases seen in only a single experiment at the two lower test concentrations in the presence of S9-mix activation in strain TA102 were not reproducible or concentration-related and were therefore considered to be chance occurrences and not related to treatment with 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] (King, 2000). It was concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to either the limit of toxicity or 5000 μg/plate (the maximum recommended concentration, according to current regulatory guidelines), in the absence and in the presence of a rat liver metabolic activation system (S9-mix).

Micronucleus Assay

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

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

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

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

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

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

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

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

Conclusion

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

For a summary of *in vitro / in vivo* genotoxicity data considered by the EFSA in FGE.207, see Table 7.

4.5. EFSA Considerations

The Panel concluded, based on the genotoxicity data available for substances in FGE.05Rev2 and substances [FL-no: 05.020, 05.124 and 09.931] (FGE.202 and FGE.207) that genotoxicity is not of concern for all the 23 substances in FGE.72Rev1.

5. Application of the Procedure

5.1. Application of the Procedure to 23 Aliphatic Branched-chain Saturated and Unsaturated Alcohols, Aldehydes, Acids and related Esters by JECFA (JECFA, 2004b)

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

The JECFA concluded 22 of the aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural class I (step A3).

The remaining substance, citral [FL-no: 05.020], is not endogenous. The evaluation therefore proceeded to step A5. A NOAEL of 60 mg/kg bw from the carcinogenicity study (NTP, 2003) exists to provide an adequate margin of safety to the estimated intake as flavouring substance.

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

The stepwise evaluations of the 23 substances evaluated by the JECFA are summarised in Table 8.

5.2. Application of the Procedure to 37 Branched- and Straight-chain Unsaturated Carboxylic Acids and Esters of These with Aliphatic Saturated Alcohols Evaluated by EFSA in FGE.05Rev2 (EFSA CEF Panel, 2010)

Thirty-seven candidate substances were evaluated in FGE.05Rev2. Thirty-four substances are classified into structural class I and three substances into structural class II using the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

All 37 substances were concluded at step A3 – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intake is below the threshold for the structural class (step A3).

In conclusion the Panel evaluated all 37 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The stepwise evaluations of the 37 substances evaluated by the EFSA are summarised in Table 9.

5.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all 23 substances in the group of aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters.

CONCLUSION

The JECFA evaluated a group of 23 aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters at the 61st meeting.

The Panel concluded that the 23 substances are structurally related to the group of branched- and straight-chain unsaturated carboxylic acids and esters of these with aliphatic saturated alcohols evaluated by EFSA in the Flavouring Group Evaluation 05, Revision 2 (FGE.05Rev2).

The Panel concluded, based on the genotoxicity data available for substances in FGE.05Rev2 and substances [FL-no: 05.020, 05.124 and 09.931] (FGE.202 and FGE.207), that genotoxicity is not of concern for all the 23 substances in FGE.72Rev1.

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all 23 substances considered in this FGE.

For all 23 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.

In order to determine whether the conclusion for the 23 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 are available for all 23 JECFA evaluated substances.

Thus, for all 23 JECFA-evaluated aliphatic branched-chain saturated and unsaturated alcohols, aldehydes, acids and related esters [FL-no: 02.011, 02.012, 02.027, 02.029, 02.058, 02.076, 02.109, 05.020, 05.021, 05.124, 05.148, 05.169, 08.036, 08.044, 08.047, 08.055, 08.064, 08.070, 08.079, 09.273, 09.408, 09.931 and 16.001] the Panel agrees with JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

SUMMARY OF GENOTOXICITY DATA

Table 2: Genotoxicity Data (*in vitro / in vivo*) evaluated by JECFA (JECFA, 2004b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
In vitro						
02.011 1219	Citronellol	Reverse mutation	*S. typhimurium* TA98 and TA100	0.05 - 100 µl/plate (0.04 - 85 µg/plate)	Negative[a]	(Rockwell and Raw, 1979)
		Rec assay	*B. subtilis* M45 and H17	17 µg/disk	Negative	(Oda et al., 1979)
02.012 1223	Geraniol	Reverse mutation	*S. typhimurium* TA100	0.01 - 1.0 µl (8.89 - 889 mg/tube)	Negative[b]	(Eder et al., 1980)
			S. typhimurium TA98, TA100, TA1535, TA1537	3 µmol/plate (463 µg/plate)	Negative[b]	(Florin et al., 1980)
			S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	≤500 µg/plate	Negative[a]	(Ishidate et al., 1984)
		Sister chromatid exchange	Chinese hamster ovary cells	33.3 - 333 µmol/l (5.14 - 51.4 µg/ml)	Negative[c]	(Sasaki et al., 1989)
		Chromosomal aberration	Chinese hamster fibroblast cells	≤125 µg/ml	Negative[c,d]	(Ishidate et al., 1984)
		Rec assay	*B. subtilis* M45 and H17	16 µg/disk	Negative	(Oda et al., 1979)
02.029 1230	3,7,11-Trimethyldodeca-2,6,10-trien-1-ol	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	8 - 5000 µg/plate	Negative[b]	(Creutziger, 1989)
			S. typhimurium TA98, TA100, TA1535, TA1537	3 µmol/plate (667 µg/plate)[e]	Negative[b]	(Florin et al., 1980)
05.020	Citral	Reverse mutation	*S. typhimurium* TA98, TA100, TA97a, TA102	5 - 700 µg/plate	Negative[b]	(Gomes-Carneiro et al., 1998)
			S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	Up to 100 µg/plate	Negative[a]	(Ishidate et al., 1984)
			S. typhimurium TA100	NR	Negative[b]	(Lutz et al., 1982)
			S. typhimurium TA98, TA100,	1 - 160 µg/plate	Negative[b]	(Zeiger et al., 1987)

Table 2: Genotoxicity Data (*in vitro / in vivo*) evaluated by JECFA (JECFA, 2004b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
			TA1535, TA1537			(NTP, 2003)
		Mutation	*E. coli* WP2uvrA (trp^-)	13 - 100 µg/plate	Negative	(Yoo, 1986)
		Sister chromatid exchange	Chinese hamster ovary cells	0.289 - 40.2 µg/ml	Positive[b]	(NTP, 2003)
		Chromosomal aberration	Chinese hamster ovary cells	12.5 - 60.6 µg/ml	Negative[b]	(NTP, 2003)
			Chinese hamster fibroblast cells	Up to 30 µg/ml	Negative[c]	(Ishidate et al., 1984)
		Rec assay	*B. subtilis* M45 and H17	17 µg/disk	Negative	(Oda et al., 1979)
			B. subtilis M45 and H17	0.16, 0.32, 0.63 µl/disk (142, 284, 560 µg/disk) 1.25, 2.5 µl/disk (1110, 2220 µg/disk)	Negative Positive	(Kuroda et al., 1984)
			B. subtilis M45 and H17	< 2.5 µl/disk (< 2220 µg/disk)	Positive	(Yoo, 1986)
		Induction of tumour suppressor protein p53 (DNA damage)	Mouse fibroblast cells (NTCT 929)	10 - 30 µg/ml	Positive	(Duerksen-Hughes et al., 1999)
05.021 1220	Citronellal	Reverse mutation	*S. typhimurium* TA98, TA100, TA97a, TA102	1 - 300 µg/plate	Negative[b]	(Gomes-Carneiro et al., 1998)
			S. typhimurium TA98 and TA100	0.05 - 500 µg/plate	Negative[b]	(Kasamaki et al., 1982)
		Sister chromatid exchange	Chinese hamster ovary cells	3.3 - 100 µmol/l (0.51 - 15.4 µg/ml)	Negative[c]	(Sasaki et al., 1989)
		Chromosomal aberration	Chinese hamster B241 cells	50 nmol/l (0.008 µg/ml)	Positive[b]	(Kasamaki et al., 1982)
		Rec assay	*B. subtilis*	17 µg/disk	Negative	(Oda et al., 1979)

Table 2: Genotoxicity Data (*in vitro / in vivo*) evaluated by JECFA (JECFA, 2004b)

FL-no JECFA-no	EU Register name JECFA name	End-point	Test system	Concentration	Results	Reference
			M45 and H17			
In vivo						
05.020 1225	Citral	Micronucleus formation	Mouse bone marrow erythrocytes	250, 500 or 750 mg/kg bw[f]	Negative	(NTP, 2003)
			Mouse peripheral blood erythrocytes	745, 1840, 3915 or 8110 mg/kg bw per day (males)[g]	Negative	(NTP, 2003)
				790, 1820, 3870 or 7550 mg/kg bw per day (females)[g]	Negative	(NTP, 2003)

a With metabolic activation.
b With and without metabolic activation.
c Without metabolic activation.
d Polyploidy (8 %) was observed at the highest dose tested.
e Substance precipitated on the plate.
f Three intraperitoneal injections given at 24-hour intervals; male mice only.
g Microencapsulated citral was administered in the diet for 14 weeks.

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.05Rev2 (EFSA CEF Panel, 2010)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Oleic acid [08.013])	Ames	*S. typhimurium* TA1535, TA1537, TA98, TA100, TA1538. *E. coli*	5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	Modified Ames, reincubation.
		S. typhimurium TA1535, TA98, TA100, TA1537	333 µg/plate	Negative[1]	(Mortelmans et al., 1986)	Modified Ames, reincubation.
	Rec assay	*B. subtilis*	1.0 mg/plate	Negative[1]	(Osawa and Namiki, 1982)	
	Sister Chromatid Exchange	CH V79	2.5 - 10 µg/ml	Negative	(Kinsella, 1982)	
	Chromosome aberrations	CH V79	2.5 - 10 µg/ml	Positive	(Kinsella, 1982)	No data on cytotoxicity reported.
	6-TG resistance	CH V79	1.0 µg/ml	Negative	(Kinsella, 1982)	
Methyl oleate [09.652]	Ames	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0.100, 0.333, 3.333 and 10 mg/plate	Negative[1]	(Mortelmans et al., 1986)	
(Methyl linoleate [09.646] & Methyl linolenate (mixture)	Ames (His reversion)	*S. typhimurium* TA100, TA98, TA102, TA97, TA1537	1.0 mg/plate	Negative[1]	(MacGregor et al., 1985)	Tests were conducted with methyl linoleate and methyl linolenate separately, with the same result.
(2,6-Dimethyl-5-heptenal [05.074])	Ames	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate	Negative[1]	(Wild et al., 1983)	
	Ames	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	50 mg/plate	Negative[1]	(Heck et al., 1989)	
(2,6-Dimethyl-5-heptenal [05.074])	UDS	Rat hepatocytes	1.0 mg/ml	Negative[1]	(Heck et al., 1989)	
Methyl methacrylate [09.647]	Ames	*S. typhimurium* TA98, TA100, TA1535, TA1537	As part of a bonecement extract	Negative[1]	(Jensen et al., 1991)	
		S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	150 - 4700 µg/plate	Negative[1]	(Hachitani et al., 1982)	The study cannot fully be evaluated as text is in Japanese, however, from the tables reported the result

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.05Rev2 (EFSA CEF Panel, 2010)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						seems to be valid.
		S. typhimurium TA98, TA100, TA1535, TA1538	100, 1000 and 9000 ppm (tested as a gas)	Negative[1]	(Anderson et al., 1979; Rohm & Haas Co., 1976a)	
		S. typhimurium TA97, TA98, TA100, TA1535, TA1537	10 - 10000 µg/plate	Negative[1]	(Zeiger, 1990)	
		S. typhimurium TA98, TA100, TA1535, TA1538	4 - 2500 µg/plate	Negative[1]	(ICI, 1976a)	
		S. typhimurium TA1535, TA1537, TA1538	10 mg/plate	Negative[1]	(DuPont, 1975)	
		S. typhimurium TA98, TA100, TA1535, TA1537	1000, 2500, 5000, 7500 and 10000 µg/plate	Negative[1]	(DuPont, 1979b)	
		S. typhimurium TA100	10, 25 and 50 mM (liquid suspension assay)	Weak positive[1]	(DuPont, 1979b)	Cytotoxicity at all dose levels ranging from 21 - 58 % survival at low-dose level and 18 - 36 % survival at high-dose level.
		S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate	Negative[1]	(Lijinsky & Andrews, 1980)	
		S. typhimurium TA97, TA98, TA100, TA1535	33 - 10000 µg/plate	Negative[1]	(NTP, 1986)	
		S. typhimurium TA98, TA1535, TA1537, TA1538	40 - 10000 µg/plate	Negative[1]	(Waegemaekers and Bensink, 1984)	
		S. typhimurium TA100	100 - 10000 µg/2 ml	Negative[1]	(Waegemaekers and Bensink, 1984)	
		S. typhimurium TA97a, TA98, TA100, TA102, TA104	0.005 - 25 mg/plate (tested eluates in DMSO and saline; 100 µl of eluate is expressed as 5 mg/plate)	Negative	(Schweikl et al., 1994)	

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.05Rev2 (EFSA CEF Panel, 2010)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		S. typhimurium TA98, TA100, TA1537	0.08 - 2.5 %	Negative[1]	(DuPont, 1979a)	
		S. typhimurium TA1535	0.08 - 2.5 %	Negative[2] Weak positive[3]	(DuPont, 1979a)	Weak pos.: The dose levels selected for the test were nontoxic or only slightly toxic.
		S. typhimurium TA100	25 mM (suspension assay)	Negative [2] Weak positive[3]	(DuPont, 1979a)	Survival at 25 mM was 28 - 29 %.
	Forward mutation	*S. typhimurium* TM677	10 - 100 mM	Weak positive[2] Negative[3]	(Poss et al., 1979)	Relative survival was 0.50 at 10 mM and 0.10 at 100 mM.
		S. typhimurium TM677	25 - 50 mM	Weak positive[2]	(Haskell Laboratory, 1989)	Slight increase in mutagenicity, but percent survival was only 20 – 22 % at low-dose level and 12 - 17 % at high-dose level.
	Chromosome aberrations	CHO	5000 µg/ml (50 mM)	Weak positive[2]	(Anderson et al., 1990) (NTP, 1986)	Increase in percentage of aberrant cells was only at concentrations above 10 mM; no cytotoxicity data reported.
		CHO	1600 µg/ml (16 mM)	Positive	(Anderson et al., 1990) (NTP, 1986)	Increase in percentage of aberrant cells was only at concentrations above 10 mM; no cytotoxicity data reported.
		L5178Y TK+/- cells	2200 - 3000 µg/ml	Weak positive[3]	(Doerr et al., 1989)	Survival was 26 % at 2200 µg/ml and 12 % at 3000 µg/ml.
	Mouse lymphoma	L5178Y TK+/- cells	500 µg/ml (5 mM)	Positive[2]	(Amtower et al., 1986)	No data on cytotoxicity available.
		L5178Y TK+/- cells	1000 - 3000 µg/ml (10 - 30 mM)	Positive[3]	(Moore et al., 1988)	Mutagenic responses and increases of small colonies were small, not clearly dose-related and observed only at concentrations above 10 mM. Dose-dependent effects on survival, with 60 % survival at 1000 µg/ml; approximately 15 % survival at 3000 µg/ml.

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.05Rev2 (EFSA CEF Panel, 2010)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		L5178Y TK+/- cells	2200 – 3000 µg/ml (22 – 30 mM)	Positive[3]	(Doerr et al., 1989)	Increases of mutation frequencies occurred only at concentrations above 10 mM. There was a higher than normal level of small colonies in the control cultures. Dose-dependent effects on survival, with 53 % survival at 1000 µg/ml and 12 % at 3000 µg/ml.
		L5178Y TK+/- cells	500 - 1000 µg/ml (5 – 10 mM)	Positive[2]	(Dearfield et al., 1991)	Percent survival was approximately 80 % at 500 µg/ml and approximately 40 % at 1000 µg/ml.
		L5178Y TK+/- cells	1500 - 3000 µg/ml (15 - 30 mM)	Positive[3]	(Dearfield et al., 1991)	Percent survival was approximately 50 % at 1500 µg/ml and approximately 15 % at 3000 µg/ml.
		L5178Y TK+/- cells	300 nl/ml (cytotoxic conc.) 100 nl/ml	Positive[2] Negative[3]	(Rohm & Haas Co., 1985)	
		L5178Y TK+/- cells	0.125 - 1 µl/ml	Positive[2] Ambiguous[3]	(NTP, 1986)	Ambiguous: Small but dose-related increases in mutant frequencies and numbers, but dose-related cytotoxicity was observed.
		L5178Y TK+/- cells	≥ 200 nl/ml 500 - 1500 nl/ml	Positive[1]	(Myhr et al., 1990)	Treatments of 1500 nl/ml (without activation) and 2000 nl/ml (with activation) considered extremely toxic and/or lethal. No other cytotoxicity data available.
	SCE	Human lymphocytes	0.1 µg/ml	Negative[3]	(Cannas et al., 1987; Bigatti et al., 1989)	
		CHO	16 - 5000 µg/ml	Ambiguous[1]	(Anderson et al., 1990)	Small increases in SCE frequency were reported.
	HRPT	CH V79 B cells	10 - 20 µg/ml	Very weak positive[3]	(Schweikl et al., 1998)	Survival was 71 % and 49 % at 10 and 20 µg/ml, respectively.
	Cell transformation	BHK21/C13 cells	0.01 - 0.000001 M	Negative	(Anderson et al., 1979)	

Table 3: Genotoxicity Data (*in vitro*) EFSA / FGE.05Rev2 (EFSA CEF Panel, 2010)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Micronucleus	Binucleated L5178Y cells	2200 - 3000 µg/ml (22 - 30 mM)	Ambiguous	(Doerr et al., 1989)	Small but dose-related increases in mutant frequencies and numbers. Small but not clearly dose related increases in frequencies of micro-nucleated cells. No cytotoxicity data reported.
Ethyl methacrylate [09.375]	Ames	*S. typhimurium* TA98, TA100, TA1535, TA1537	33 - 10000 µg/plate	Negative[1]	(Zeiger et al., 1987)	
		S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	40 - 2500 µg/plate	Negative[1]	(Waegemaekers and Bensink, 1984)	
	Mouse lymphoma	L5178Y TK+/- cells	> 1400 µg/ml	Weak Positive[3]	(Moore et al., 1988)	Negative at 1400 µg/ml and below; survival at 1400 µg/ml and above ranged from 2 % to 33 %, with cytotoxicity appearing to reach a plateau at concentrations above 1500 µg/ml.
	SCE	CHO	NR	Positive	(NTP, 1987)	Abstract in table format only, study report not available for re-evaluation.
Isobutyl 2-methylprop-2-enoate [09.586]	Ames	*S. typhimurium* TA98, TA100, TA1535, TA1537	100, 333, 1000 and 10000 µg/plate	Negative[1]	(Zeiger et al., 1987)	

1 With and without metabolic activation.
2 With metabolic activation.
3 Without metabolic activation.

Table 4: Genotoxicity Data (*in vivo*) for FGE.05Rev2 (EFSA CEF Panel, 2010)

Chemical Name [FL-no]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(2,6-Dimethyl-5-heptenal [05.074])	Micronucleus	Mouse (bone marrow)	IP injection	Single dose of 0, 420, 980 and 1540 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	Basc test (Sex-linked recessive lethal mutation)	*D. melanogaster*	Diet	25 mM	Negative	(Wild et al., 1983)	A single dose was tested in two experiments. Method not described in detail.
Methyl methacrylate [09.647]	Micronucleus	Mouse (bone marrow)	Gavage	Single dose of 1130 to 4520 mg/kg or 4 doses of 1130 mg/kg	Negative	(Hachitani et al., 1982)	The study cannot be evaluated as text is in Japanese. Thus, e.g. it is not clear if samples were taken at different sampling times after single treatment and if sampling time was adequate after multiple treatment. Frequency of reticulocytes only slightly changed compared to control. Therefore it is not clear whether the substance had reached the bone marrow.
		Mouse (bone marrow)	IP injection	Single dose of methacrylate bone cement mixture	Negative	(Jensen et al., 1991)	Not relevant since an extract of a mixture (containing some additives used as accelerator, stabiliser, colourings etc.) was tested.
	Sex-linked recessive lethal mutation	*D. melanogaster*	Inhalation	1400 ppm	Negative	(Foureman et al., 1994)	Sufficient experimental details reported. Result is considered as valid.
		D. melanogaster	Inhalation.	14000 ppm	Negative	(Foureman et al., 1994)	Sufficient experimental details reported. Result is considered as valid.
	Dominant lethal	Mouse	Inhalation, 6 hours/day for 5 days	100, 1000 and 9000 ppm	Negative	(ICI, 1976b)	Unpublished non-GLP study. Report contains sufficient details. Result is considered as valid.
	SCE	Human (38 male workers)	Inhalation; 8 hours/day	0.9 - 71.9 ppm	Negative	(Seiji et al., 1994)	Exposure period was not reported. 11 unexposed subjects were used as controls.

Table 4: Genotoxicity Data (*in vivo*) for FGE.05Rev2 (EFSA CEF Panel, 2010)

Chemical Name [FL-no]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							A marginal increase was found (6.11 vs. 4.91 SCEs/cell). However, this effect was considered to be age-related (and not dependent on methyl methacrylate exposure). Result is considered as valid.
	Chromosome aberrations	Human (38 male workers)	Inhalation; 8 hours/day	0.9 - 71.9 ppm	Negative	(Seiji et al., 1994)	Exposure period was not reported. 11 unexposed subjects were used as controls. Result is considered as valid.
		Rat (bone marrow)	Inhalation, single 2 hours exposure or 5 hours/day for 5 days	100 - 9000 ppm	Weak positive	(Rohm & Haas Co., 1976b; Rohm & Haas Co., 1979)	"Both studies suffer from inadequate description; esp. the second study demonstrates severe methodological problems, e.g., analysis of 50 metaphases was not possible for 10 out of 27 animals in the acute and 10 out 26 in the subacute test. Altogether, a clear conclusion cannot be drawn from these studies." (CEC 2002).
Isobutyl 2-methyl-prop-2-enoate [09.586]	Micronucleus	Mouse	Gavage	5000 mg/kg	Negative	(Roehm GmbH., 1989)	Reported to be in accordance with OECD Guideline 474, however, the study cannot be re-evaluated as only a summary of the EU-IUCLID database is available. According to this summary, a decrease of PCE/NCE ratio was observed. This indicates that the substance had reached the target cells.

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.202 (EFSA, 2009b)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments
Citral [05.020]	Reverse mutation	*S. typhimurium* TA98, TA100, TA97a, TA102	5 - 700 µg/plate	Negative[a]	(Gomes-Carneiro et al., 1998)	Published non-GLP study containing sufficient details. Result is considered as valid.
		S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	Up to 100 µg/plate	Negative[b]	(Ishidate et al., 1984)	Valid. According to current guidelines. The study is considered valid.
		S. typhimurium TA100	NR	Negative[a]	(Lutz et al., 1982)	Validity cannot be evaluated. One strain only, Concentrations tested not specified. no re-run of the test; no other data on experimental results or design apart from a description of the test method.
		S. typhimurium TA98, TA100, TA1535, TA1537	1 - 160 µg/plate	Negative[a]	(Zeiger et al., 1987) (NTP, 2003)	Valid. Standard NTP study carried out according to US EPA Guidelines; result is considered valid.
	Mutation	*E. coli* WP2uvrA (trp -)	13 - 100 µg/plate	Negative	(Yoo, 1986)	Validity cannot be evaluated (study in Japanese).
	Sister chromatid exchange	Chinese hamster ovary cells	0.289 - 40.2 µg/ml	Positive[a]	(NTP, 2003)	Valid. Standard NTP study carried out according to US EPA Guidelines; result is considered valid.
	Chromosomal aberration	Chinese hamster ovary cells	12.5 - 60.6 µg/ml	Negative[a]	(NTP, 2003)	Valid. Standard NTP study carried out according to US EPA Guidelines; result is considered valid.
		Chinese hamster fibroblast cells	Up to 30 µg/ml	Negative[c]	(Ishidate et al., 1984)	Limited validity (performed only in the presence of metabolic activation). Study of limited validity.
	Rec assay	*B. subtilis* M45 and H17	17 µg/disk	Negative	(Oda et al., 1979)	The test system used is considered inappropriate; insufficient validity.
		B. subtilis M45 and H17	0.16, 0.32, 0.63 µl/disk (142, 284, 560 µg/disk)[d] 1.25, 2.5 µl/disk (1110, 2220 µg/disk)[d]	Negative Positive	(Kuroda et al., 1984)	Validity cannot be evaluated. Article in Japanese; with limited information in tables and abstract. Assay of limited relevance.

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.202 (EFSA, 2009b)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments
	Rec assay	*B. subtilis* M45 and H17	< 2.5 µl/disk (< 2220 µg/disk)	Positive	(Yoo, 1986)	Validity cannot med evaluated (study in Japanese). Study of limited relevance.
	Induction of tumour suppressor protein p53 (DNA damage)	Mouse fibroblast cells (NTCT 929)	10 - 30 µg/ml	Positive	(Duerksen-Hughes et al., 1999)	The Induction of tumor suppressor protein p53 may be considered as indicator for genotoxicity. Result is considered valid, however, it has only limited relevance.
3-methyl-2-butenal [05.124]	Ames test (preincubation)	*S. typhimurium* TA98, TA100		Positive[a]	(BASF, 1991)	Valid. Modified Ames test: Unpublished non-GLP study, carried out in accordance with the OECD Guideline 471. The study contains sufficient details and is considered valid.

NR Not reported.
a With and without metabolic activation.
b With metabolic activation.
c Without metabolic activation.
d Calculated using a density of 0.888 (Merck Index, 1997).
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 inappropriate / not validated test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided, text not in a Community language).

Table 6: Genotoxicity Data (*in vivo*) from FGE.202 (EFSA, 2009b)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments a)
Citral [05.020]	Micronucleus formation	Mouse bone marrow erythrocytes	Three intraperitoneal injections given at 24-hour intervals; male mice only	250, 500, or 750 mg/kg bw	Negative	(NTP, 2003)	NTP study carried out according to US-EPA Guideline. Result is considered as valid.
		Mouse peripheral blood erythrocytes	Microencapsulated citral was administered in the diet for 14 weeks	745, 1840, 3915, or 8110 mg/kg bw per day (males) 790, 1820, 3870, or 7550 mg/kg bw per day (females)	Negative Negative	(NTP, 2003)	NTP study carried out according to a non-standard guideline; result is considered of limited validity.
3-methyl-2-butenal [05.124]	UDS	Rat hepatocytes	Oral administration	350 and 700 mg/kg body weight	Negative	(BASF, 2001)	Unpublished GLP study, carried out in accordance with OECD Guideline 486. The study is considered valid.
	Micronucleus test	Mouse bone marrow erythrocytes	Oral administration	175, 350 and 750 mg/kg body weight	Negative	(BASF, 1992)	Unpublished GLP study, carried out in accordance with OECD Guideline (1991). The study is considered valid.

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 inappropriate / not validated test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided, text not in a Community language).

Table 7: Genotoxicity Data (*in vitro*) from FGE.207 (EFSA CEF Panel, 2013)

Chemical Name [FL-no:]	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [09.931]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	5 - 1500 µg/plate[1,3]; 5 - 5000 µg/plate[2,3]	Negative[1,3]; Equivocal[2,3]	(King, 2000)	Reliable without restriction. GLP study in compliance with OECD Guideline 471. A small increase in TA102 revertant numbers was seen at 15 and 50 µg/plate in the presence of S9-mix, but not at higher concentrations.
		S. typhimurium TA98, TA100, TA1535, TA1537 and TA102	5 - 1500 µg/plate[1,3]; 5 - 5000 µg/plate[2,3]	Negative[1,3]; Negative[2,3]		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 µg/plate in the presence of S9-mix was not reproduced in the second experiment.
		S. typhimurium TA102	5 - 1500 µg/plate[2,3]	Negative		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 µg/plate in the presence of S9-mix was not reproduced in the third experiment.
	Micronucleus Assay	Human peripheral blood lymphocytes (Male Donors)	70 - 120 µg/ml [1,4]; 120 - 225 µg/mL [2,4]; 20 - 60 µg/mL [1,5]; 119.2 - 290 µg/mL [2,4]	Weak positive +S9; Re-test within normal values	(Whitwell, 2012)	Reliable without restriction. GLP study in compliance with OECD Guideline 487. Weak evidence of inducing micronuclei in the presence of S9-mix in a first experiment (increases only in one culture). A re-test under the same conditions and using a higher top concentration resulted in MNBN frequencies within the historical negative control range at 95th percentile, but were statistically significant due to low vehicle control values.

[1] Without S9-mix metabolic activation.
[2] With S9-mix metabolic activation.
[3] Plate incorporation method.
[4] 3-hour incubation with 21-hour recovery period.
[5] 24-hour incubation with no recovery period.

SUMMARY OF SAFETY EVALUATIONS

Table 8: Summary of Safety Evaluation by the JECFA (JECFA, 2004b)

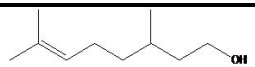
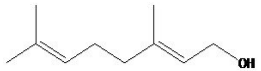
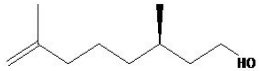
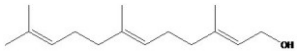
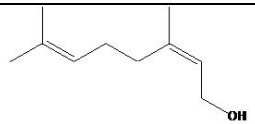
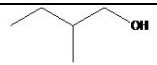
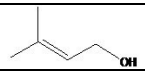
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [1] US MSDI (µg/*capita*/day)	Class [2] Evaluation procedure path [3]	Outcome on the named compound [4] or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.011 1219	Citronellol		320 0.5	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
02.012 1223	Geraniol		550 315	Class I A3: Intake below threshold	4)	Evaluated in FGE.202, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
02.027 1222	Rhodinol		13 8.4	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
02.029 1230	3,7,11-Trimethyldodeca-2,6,10-trien-1-ol		7.7 2.6	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
02.058 1224	Nerol		250 171	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
02.076 1199	2-Methylbutan-1-ol		0.73 35	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
02.109 1200	3-Methylbut-2-en-1-ol		4.6 3.8	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake	No safety concern at the estimated level

Table 8: Summary of Safety Evaluation by the JECFA (JECFA, 2004b)

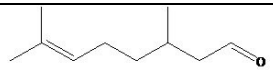
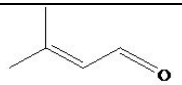
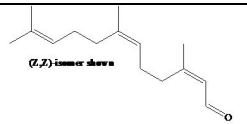
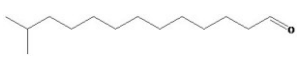
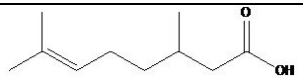
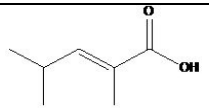
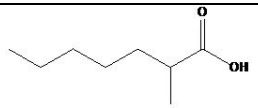
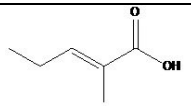
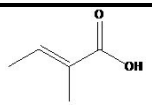
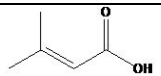
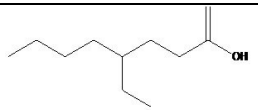
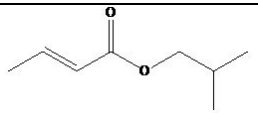
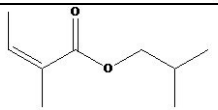
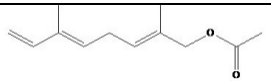
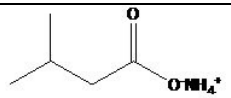
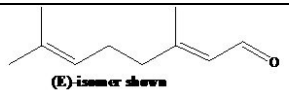
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [1)] US MSDI (µg/*capita*/day)	Class [2)] Evaluation procedure path [3)]	Outcome on the named compound [4)] or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
						based on the MSDI approach.	of intake based on the MSDI approach.
05.021 1220	Citronellal		810 324	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
05.124 1202	3-Methylcrotonaldehyde		3.3 0.5	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
05.148 1228	Farnesal		0.49 0.2	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
05.169 1229	12-Methyltridecanal		0.24 0.5	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
08.036 1221	Citronellic acid		2.7 0.2	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
08.044 1211	2,4-Dimethylpent-2-enoic acid		0.12 0.1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
08.047 1212	2-Methylheptanoic acid		14 6	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
08.055 1210	2-Methyl-2-pentenoic acid		36 20	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI	No safety concern at the estimated level of intake based on

Table 8: Summary of Safety Evaluation by the JECFA (JECFA, 2004b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [1] US MSDI (µg/*capita*/day)	Class [2] Evaluation procedure path [3]	Outcome on the named compound [4] or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
						approach.	the MSDI approach.
08.064 1205	2-Methylcrotonic acid		4.1 1.6	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
08.070 1204	3-Methylcrotonic acid		0.012 0.01	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
08.079 1218	4-Ethyloctanoic acid		0.73 4	Class I A3: Intake below threshold	4)	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.
09.273 1206	Isobutyl crotonate		0.46 45	Class I A3: Intake below threshold	4)	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.
09.408 1213	Isobutyl 2-methylbut-2(cis)-enoate		0.12 0.1	Class I A3: Intake below threshold	4)	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.
09.931 1226	2,6-Dimethyl-2,5,7-octatriene-1-ol acetate		1.2 7.7	Class I A3: Intake below threshold	4)	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.
16.001 1203	Ammonium isovalerate		15 16	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
05.020 1225	Citral		5844 6990	Class I A3: Intake above threshold A4: Not endogenous A5: Adequate NOAEL exists	4)	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.

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.

ND not determined

Table 9: Summary of Safety Evaluation by the EFSA (FGE.05Rev2) (EFSA CEF Panel, 2010)

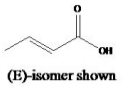
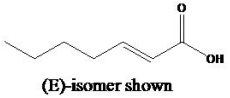
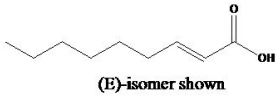
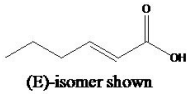
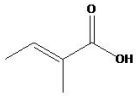
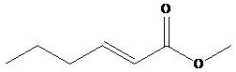
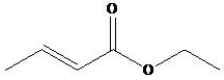
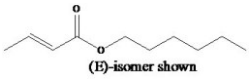
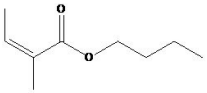
FL-no JECFA-no	EU Register name	Structural formula	MSDI [1] (µg/capita/day)	Class [2] Evaluation procedure path [3]	Outcome on the named compound [4] or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
08.072	But-2-enoic acid (cis and trans)	 (E)-isomer shown	4.0	Class I A3: Intake below threshold	4)	7)	
08.083	Hept-2-enoic acid	 (E)-isomer shown	6.1	Class I A3: Intake below threshold	4)	7)	
08.101	Non-2-enoic acid	 (E)-isomer shown	6.1	Class I A3: Intake below threshold	4)	7)	
08.119	2-Hexenoic acid	 (E)-isomer shown	240	Class I A3: Intake below threshold	4)	7)	
08.120	2-Methyl-2-butenoic acid		6.1	Class I A3: Intake below threshold	4)	7)	
09.181	Methyl hex-2-enoate		0.037	Class I A3: Intake below threshold	4)	7)	
09.248	Ethyl trans-2-butenoate		12	Class I A3: Intake below threshold	4)	6)	
09.266 1807	Hexyl 2-butenoate	 (E)-isomer shown	0.12	Class I A3: Intake below threshold	4)	6)	
09.321	Butyl 2-methylbut-2(cis)-enoate		1.2	Class I A3: Intake below threshold	4)	6)	

Table 9: Summary of Safety Evaluation by the EFSA (FGE.05Rev2) (EFSA CEF Panel, 2010)

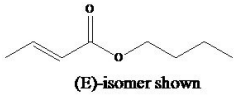
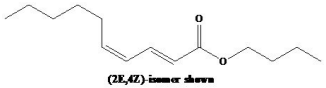
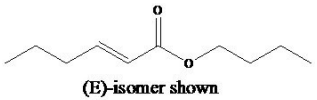
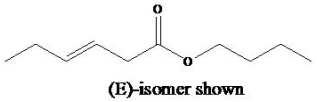
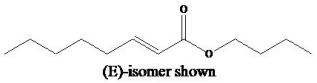
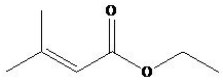
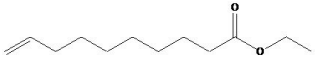
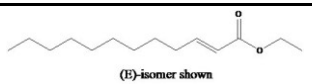
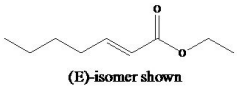
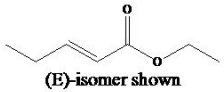
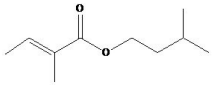
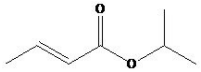
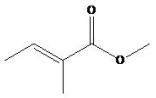
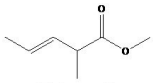
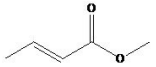
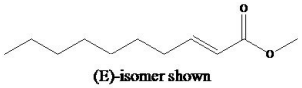
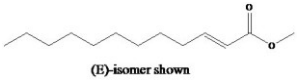
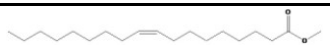
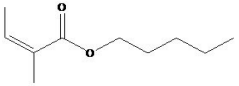
FL-no JECFA-no	EU Register name	Structural formula	MSDI [1] (μg/capita/day)	Class [2] Evaluation procedure path [3]	Outcome on the named compound [4] or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.324	Butyl but-2-enoate	 (E)-isomer shown	1.7	Class I A3: Intake below threshold	4)	6)	
09.326	Butyl deca-2,4-dienoate	 (2E,4Z)-isomer shown	0.0012	Class I A3: Intake below threshold	4)	6)	
09.329	Butyl hex-2-enoate	 (E)-isomer shown	1.0	Class I A3: Intake below threshold	4)	7)	
09.330	Butyl hex-3-enoate	 (E)-isomer shown	0.12	Class I A3: Intake below threshold	4)	6)	
09.335	Butyl oct-2-enoate	 (E)-isomer shown	0.66	Class I A3: Intake below threshold	4)	7)	
09.365	Ethyl 3-methylcrotonate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.370	Ethyl dec-9-enoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.372	Ethyl dodec-2-enoate	 (E)-isomer shown	0.34	Class I A3: Intake below threshold	4)	6)	
09.374	Ethyl hept-2-enoate	 (E)-isomer shown	0.61	Class I A3: Intake below threshold	4)	6)	

Table 9: Summary of Safety Evaluation by the EFSA (FGE.05Rev2) (EFSA CEF Panel, 2010)

FL-no JECFA-no	EU Register name	Structural formula	MSDI [1] (μg/capita/day)	Class [2] Evaluation procedure path [3]	Outcome on the named compound [4] or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.379	Ethyl pent-2-enoate	 (E)-isomer shown	0.037	Class I A3: Intake below threshold	4)	7)	
09.596	Isopentyl 2-methylcrotonate		0.012	Class I A3: Intake below threshold	4)	6)	
09.603	Isopropyl crotonate		0.24	Class I A3: Intake below threshold	4)	6)	
09.624	Methyl 2-methylcrotonate		0.12	Class I A3: Intake below threshold	4)	6)	
09.625	Methyl 2-methylpent-3-enoate	 (E)-isomer shown	0.0012	Class I A3: Intake below threshold	4)	6)	
09.636	Methyl crotonate		0.12	Class I A3: Intake below threshold	4)	6)	
09.637	Methyl dec-2-enoate	 (E)-isomer shown	0.37	Class I A3: Intake below threshold	4)	7)	
09.641	Methyl dodec-2-enoate	 (E)-isomer shown	0.56	Class I A3: Intake below threshold	4)	6)	
09.652	Methyl oleate		1.2	Class I A3: Intake below threshold	4)	6)	
09.680	Pentyl 2-methylisocrotonate		0.74	Class I A3: Intake below threshold	4)	6)	

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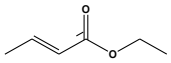
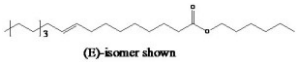
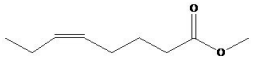
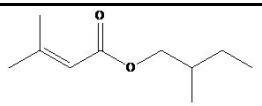
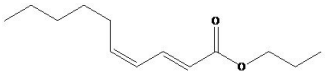
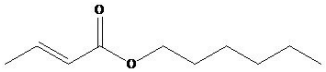
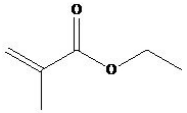
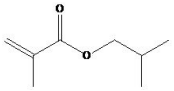
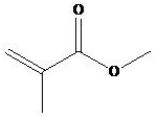
FL-no JECFA-no	EU Register name	Structural formula	MSDI [1] (µg/capita/day)	Class [2] Evaluation procedure path [3]	Outcome on the named compound [4] or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.699	Propyl crotonate		0.085	Class I A3: Intake below threshold	4)	6)	
09.865	Hexyl 9-octadecenoate	 (E)-isomer shown	0.24	Class I A3: Intake below threshold	4)	6)	
09.934 1630	Methyl (5Z)-Octenoate		3.7	Class I A3: Intake below threshold	4)	6)	
09.942	2-Methylbutyl-3-methyl-2-butenoate		1.2	Class I A3: Intake below threshold	4)	6)	
09.287	Propyl deca-2,4-dienoate	 (2E,4Z)-isomer shown	0.61	Class I A3: Intake below threshold	4)	8)	
09.578	Hexyl crotonate		2.6	Class I A3: Intake below threshold	4)	8)	
09.375	Ethyl methacrylate		0.12	Class II A3: Intake below threshold	4)	6)	
09.586	Isobutyl 2-methylprop-2-enoate		0.012	Class II A3: Intake below threshold	4)	6)	
09.647 1834	Methyl methacrylate		0.061	Class II A3: Intake below threshold	4)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.
6) No safety concern at the estimated level of intake of the material of commerce meeting the specification requirements (based on intake calculated by the MSDI approach).
7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

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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
EFFA	European Flavour and Fragrance Association
EFSA	European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
HPRT	Hypoxanthine Phosphoribosyl transferase
ID	Identity
IP	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	Micronucleated Binucleate cells
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
RI	Replication Index
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA Synthesis
WHO	World Health Organization