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EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 206 (FGE.206): Consideration of genotoxicity data on representatives for 12 alpha,beta-unsaturated ketones and precursors from chemical subgroup 1.2.3 of FGE.19 by EFSA

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 206 (FGE.206):

Consideration of genotoxicity data on representatives for 12 alpha,beta-unsaturated ketones and precursors from chemical subgroup 1.2.3 of FGE.19 by EFSA[1]

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

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

The European Food Safety Authority (EFSA) asked the Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide 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 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.

The present Flavouring Group Evaluation 206 (FGE.206), corresponding to subgroup 1.2.3 of FGE.19, concerns seven aliphatic ketones and five precursors for such ketones. The 12 substances under consideration in the present evaluation are alpha,beta-unsaturated ketone structures or can be metabolised to such, which are considered to be structural alerts for genotoxicity and the data on genotoxicity previously available did not rule out the concern for genotoxicity.

The Panel has identified two substances in subgroup 1.2.3 which will represent the other 10 substances in this subgroup. For these two substances, genotoxicity data according to the test strategy worked out by the Panel have been requested.

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1 On request from the Commission, Question No EFSA-Q-2010-01248, adopted on 25 November 2010.

2 Panel members: Arturo Anadon, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle. Correspondence: cef-unit@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Genotoxicity of Flavourings for the preparation of this Opinion: Vibe Beltoft, Mona-Lise Binderup, Wilfried Bursch, Angelo Carere, Riccardo Crebelli, Karl-Heinz Engel, Rainer Gürtler, John Christian Larsen, Wim Mennes, Karin Nørby and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific Opinion.

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Consideration of genotoxicity data on representatives for 12 alpha,beta-unsaturated ketones and precursors from chemical subgroup 1.2.3 of FGE.19 by EFSA. EFSA Journal 2011; 9(3):1922. [16 pp.]. doi:10.2903/j.efsa.2011.1922. Available online: www.efsa.europa.eu/efsajournal.htm

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The Industry has submitted data concerning genotoxicity studies for the two representative substances for this subgroup.

6-Methylhepta-3,5-dien-2-one [FL-no: 07.099] and pseudo-ionone [FL-no: 07.198] has been tested for all three genetic endpoints, gene mutations, structural and numerical chromosomal aberrations. The test compounds did not induce gene mutations in bacteria and were not clastogenic and/or aneugenic in mammalian cells *in vitro*.
The Panel concluded that the *in vitro* genotoxicity data on these two representative substances do not indicate genotoxic potential. Accordingly, all 12 substances in FGE.19 subgroup 1.2.3 are not considered to be of concern with respect to genotoxicity and will then be evaluated through the Procedure.

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KEY WORDS

FGE.19; subgroup 1.2.3, acyclic alpha,beta-unsaturated ketones with additional conjugated double-bond.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a) which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

After the completion of the evaluation programme the Union list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

Flavouring Group Evaluation 19 (FGE.19) contains 360 flavouring substances from the EU Register being alpha, beta-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008b).

The alpha, beta-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity. The Panel noted that there were limited genotoxicity data on these flavouring substances but that positive genotoxicity studies were identified for some substances in the group.

The alpha, beta-unsaturated carbonyls were subdivided into 28 subgroups on the basis of structural similarity (EFSA, 2008b). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship (Q)SAR prediction of the genotoxicity of these substances was undertaken considering a number of models (DEREKfW, TOPKAT, DTU-NFI-MultiCASE Models and ISS-Local Models, (Gry et al., 2007)).

The Panel noted that for most of these models internal and external validation has been performed, but considered that the outcome of these validations was not always extensive enough to appreciate the validity of the predictions of these models for these alpha, beta- unsaturated carbonyls. Therefore, the Panel considered it inappropriate to totally rely on (Q)SAR predictions at this point in time and decided not to take substances through the procedure based on negative (Q)SAR predictions only.

The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni & Netzeva, 2007a; Benigni & Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. Based on these data the Panel decided that 15 subgroups (1.1.1, 1.2.1, 1.2.2, 1.2.3, 2.1, 2.2, 2.3, 2.5, 3.2, 4.3, 4.5, 4.6, 5.1, 5.2 and 5.3) (EFSA, 2008b) could not be evaluated through the Procedure due to concern with respect to genotoxicity. Corresponding to these subgroups, 15 Flavouring Group Evaluations (FGEs) were established, FGE.200, 204, 205, 206, 207, 208, 209, 211, 215, 219, 221, 222, 223, 224 and 225).

For 11 subgroups the Panel decided, based on the available genotoxicity data and (Q)SAR predictions, that a further scrutiny of the data should take place before requesting additional data from the Flavouring Industry on genotoxicity. These subgroups were evaluated in FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220. For the substances in FGE.202, 214 and 218 it was concluded that a genotoxic potential could be ruled out and accordingly these substances will be evaluated using the Procedure. For all or some of the substances in the remaining FGEs, FGE.201. 203, 210, 212, 213, 216, 217 and 220 the genotoxic potential could not be ruled out.

To easy the data retrieval of the large number of structurally related alpha,beta-unsaturated substances in the different subgroups for which additional data are requested, EFSA has worked out a list of representative substances for each subgroup (EFSA, 2008bc). Likewise an EFSA genotoxicity expert group has worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008bb).

The Flavouring Industry has been requested to submit additional genotoxicity data according to the list of representative substances and test strategy for each subgroup.

The Flavouring industry has now submitted additional data and the present FGE concerns the evaluation of these data requested on genotoxicity.

TERMS OF REFERENCE

The European Commission requests the European Food Safety Authority to carry out an evaluation of the data on pseudo-ionone [FL-no: 07.198] and 6-methylhepta-3,5-dien-2-one [FL-no: 07.099], in accordance with Commission Regulation (EC) No 1565/2000. Depending on the outcome of the evaluations of these substances, the Europen Commission asks EFSA to evaluate all the substances of the corresponding subgroup (FGE.19 subgroup 1.2.3) through the Procedure.

ASSESSMENT

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

The present Flavouring Group Evaluation 206 (FGE.206), corresponding to subgroup 1.2.3 of FGE.19, concerns seven alpha,beta-unsaturated aliphatic ketones with additional double-bonds and five precursors for such ketones. The 12 substances under consideration in the present evaluation are listed in Table 1.

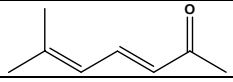
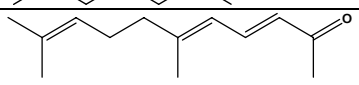
Seven of the 12 substances have previously been evaluated by the JECFA. A summary of their current evaluation status by the JECFA and the outcome of this consideration is presented in Table 2.

The alpha,beta-unsaturated aldehyde and ketone structures are considered to be structural alerts for genotoxicity (EFSA, 2008b) and the data on genotoxicity previously available did not rule out the concern for genotoxicity.

1.2. Representative Substances for Subgroup 1.2.3

The Panel has identified two substances in subgroup 1.2.3 which will represent all other 10 substances in this subgroup (EFSA, 2008bc). For these two substances genotoxicity data according to the test strategy (EFSA, 2008bb) have been requested. The representative substances are listed in Table 1.1.

TABLE 1.1 REPRESENTATIVE SUBSTANCES FOR SUBGROUP 1.2.3 OF FGE.19

FL-no JECFA-no	Subgroup	EU Register name	Structural formula	FEMA no CoE no CAS no
07.099 1134	1.2.3	6-Methylhepta-3,5-dien-2-one		3363 11143 1604-28-0
07.198 -	1.2.3	Pseudo-ionone		- 11191 141-10-6

2. Additionally Submitted Genotoxicity Data on Representative Substances of Subgroup 1.2.3

Introduction

The Industry has submitted data concerning genotoxicity studies for the two representative substances for this subgroup

• 6-Methylhepta-3,5-dien-2-one [FL-no: 07.099],

• pseudo-Ionone [FL-no: 07.198].

2.1. *In vitro* Data

In vitro genotoxicity assays have been performed on 6-methylhepta-3,5-dien-2-one and pseudo-ionone.

2.1.1. Bacterial Reverse Mutation Assay

6-Methylhepta-3,5-dien-2-one was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the presence or absence of S9. In the first experiment the concentrations tested were 1.6, 8, 40, 200, 1000 and 5000 μg/plate, and the plate incorporation methodology was used. Severe toxicity was observed at 5000 μg/plate in all strains (complete killing of bacteria). No increase in revertant colonies was observed at any of the tested concentrations. In the second experiment the concentrations were 20.5, 51.2, 128, 320, 800, 2000 and 5000 μg/plate of 6-methylhepta-3,5-dien-2-one, and treatments in the presence of S9 were carried out according to the pre-incubation method. In the absence of S9 the standard plate incorporation method was performed. Slight thinning of the bacterial lawn or complete killing of the bacteria was observed in all strains at 2000 and 5000 μg/plate in the absence of S9. In the presence of S9 cytotoxicity was observed at 800 μg/plate and above and severe toxicity (complete killing of bacteria) was observed at 5000 μg/plate in all strains (Williams, 2009). The study design complied with current recommendations (OECD 471; GLP) and an acceptable top concentration was achieved. There was no evidence of mutagenic effect induced by 6-methylhepta-3,5-dien-2-one in any of the strains, either in the absence or presence of S9. No precipitation was observed at any tested concentrations (Williams, 2009). It is concluded that under the test conditions applied, 6-methylhepta-3,5-dien-2-one is not mutagenic in bacteria.

Pseudo-ionone was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the presence or absence of S9. Evidence of toxicity was seen at concentrations of 200 μg/plate and above in the range finding study. The concentrations tested in the first experiment were 0.13, 0.64, 3.2, 16, 80, 400 and 2000 μg/plate, and plate incorporation methodology was used. Toxicity as evidenced by thinning of the bacterial lawn and/or reduction in revertant counts was observed in all strains at 400 μg/plate and above. Severe toxicity was observed at 2000 μg/plate in all strains (complete killing of bacteria). In the second experiment the concentration range was therefore modified to 12.5, 25, 50, 100, 200 or 400 μg pseudo-ionone/plate, and treatments in the presence of S9 were carried out according to the pre-incubation method. In the absence of S9 the standard plate incorporation method was performed. Evidence of toxicity was observed in all strains at 100 μg pseudo-ionone/plate through thinning of bacterial lawn, reduced revertant counts and killing of test bacteria, but sufficient data were obtained for a valid assay. Precipitation was observed in the 400 μg/plate concentration in the presence and absence of S9 in this experiment. The study design complies with current recommendations (OECD 471, GLP), and acceptable top concentrations were achieved. There was no evidence of any mutagenic effect induced by pseudo-ionone when tested up to precipitating concentrations that were clearly bactericidal (Beevers, 2009a). It is concluded that under the test conditions applied, pseudo-ionone is not mutagenic in bacteria.

2.1.2. *In vitro* Micronucleus Assays

6-Methylhepta-3,5-dien-2-one was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system. The assay was performed in accordance with OECD 487 and in compliance with GLP. In a preliminary toxicity study a wide range of concentrations up to 2000 µg/ml of 6-methylhepta-3,5-dien-2-one was tested. The highest concentration used in the main test (450 µg/ml) was limited by toxicity observed in the preliminary study. Cells were stimulated for 48 hours with phytohaemaglutinin to produce exponentially growing cells, and then treated for 3 hours (followed by 21 hours recovery) with 0, 225, 325 or 450 µg/ml of 6-methylhepta-3,5-dien-2-one in the absence of S9 and 0, 225, 300 and 350 µg/ml in the presence of S9 respectively. The levels of toxicity (reduction in replication index) at the top concentrations were 60 % and 51 % without and with S9, respectively. In a parallel assay, cells were treated for 24 hours with 0, 100, 120 or 150 µg/ml of 6-methylhepta-3,5-dien-2-one in the absence of S9 with no recovery period. The top concentration induced 56 % toxicity. There were 2 replicate cultures per treatment, and 1000 binucleate cells per replicate (i.e. 2000 cells per dose) were scored for micronuclei. No evidence of chromosomal damage or aneuploidy was observed by increased levels of micronucleated binucleate cells (MNBN) in the presence or absence of S9 metabolic activation (Whitwell, 2010a). Under the conditions of this study, 6-methylhepta-3,5-dien-2-one was not clastogenic and/aneugenic in cultured human lymphocytes.

Similarly, ***pseudo-ionone*** was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system. The assay was performed in accordance with OECD 487 and in compliance with GLP**.** The concentrations tested in the main test was based on toxicity in a preliminary toxicity study. In the main test the following concentrations were analysed:

1. 3 + 21 hour without S9: 30, 50 and 60 µg/ml
2. 3 + 21 hour with S9: 100, 110 and 120 µg/ml
3. 24 hour without S9: 10, 15 and 20 µg/ml.

Cells were stimulated for 48 hours with phytohaemaglutinin to produce exponentially growing cells, and then treated for 3 hours (followed by 21 hours recovery) with 0, 30, 50 or 60 µg/ml of pseudo-ionone in the absence of S9 and 0, 100, 110 and 120 µg/ml in the presence of S9 respectively. The levels of toxicity (reduction in replication index) at the top concentrations were 54 % and 48 % after 3 hour treatment without and with S9 respectively and 60 % after 24 hour treatment without S9. Although the target range of 50-60 % toxicity was not achieved in the presence of S9, the next highest concentration (110 µg/ml) produced 81 % toxicity, which was too high. Given the steepness of the toxicity curve, it is considered that 48 % toxicity is sufficiently close to the target range as to be acceptable. In a parallel assay, cells were treated for 24 hours with 0, 10, 15 or 20 µg/ml of pseudo-ionone in the absence of S9 with no recovery period. The top concentration induced 56 % toxicity. There were 2 replicate cultures per treatment, and 1000 binucleate cells per replicate (i.e. 2000 cells per dose) were scored for micronuclei. Treatment with pseudo-ionone under all described conditions resulted in MNBN frequencies that were comparable to and not significantly different from control groups (Lloyd, 2010).

It was noted that after 24 hours exposure in the absence of S9-mix there was a dose related increase in micronucleated cells, which was statistically significant at the highest concentration tested (20 µg/ml). The mutation frequency was 1.2 % MNBN in both cultures compared to 0.5 in the control. However this increase was within the negative control range (0.1 – 1.2) and therefore not considered as biological relevant.

Under the conditions of this study, pseudo-ionone was not clastogenic and/aneugenic in cultured human lymphocytes.

2.2. *In vivo* Data

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

2.3. Discussion of Mutagenicity/Genotoxicity Data

6-Methylhepta-3,5-dien-2-one [FL-no: 07.099] and pseudo-ionone [FL-no: 07.198] were tested for all three genetic endpoints, gene mutations, structural and numerical chromosomal aberrations. The test compounds did not induce gene mutations in bacteria and were not clastogenic and/or aneugenic in mammalian cells *in vitro*.

Although both flavouring substances showed evidence of cytotoxicity at high concentrations, they did not induce biologically significant genotoxic responses.

3. Conclusion

The Panel concluded that the *in vitro* genotoxicity data on these two representative substances do not indicate genotoxic potential. Accordingly, all 12 substances in FGE.19 subgroup 1.2.3 are not considered to be of concern with respect to genotoxicity and will then be evaluated through the Procedure.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 206 (JECFA, 2009B; JECFA, 2002D)

Table 1: Specification Summary of the Substances in the present group

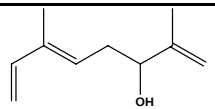
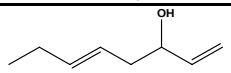
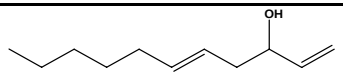
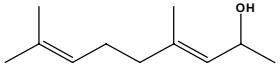
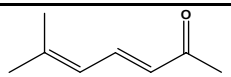
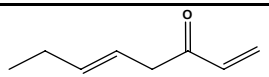
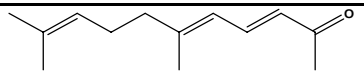
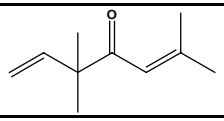
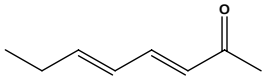
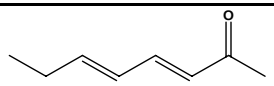
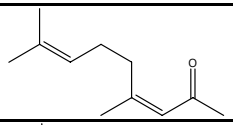
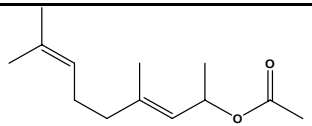
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol		- - 29414-56-0	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Freely soluble	240 n.a. MS 95 %	1.484-1.490 0.895-0.901
02.194	Octa-1,5-dien-3-ol		- - 83861-74-9	Liquid $C_8H_{14}O$ 126.20	Practically insoluble or insoluble Freely soluble	187 n.a. MS 95 %	1.441-1.447 0.832-0.838
02.211	Undeca-1,5-dien-3-ol		- - 56722-23-7	Liquid $C_{11}H_{20}O$ 168.28	Practically insoluble or insoluble Freely soluble	244 n.a. NMR 95 %	1.456-1.462 0.872-0.878
02.252 1841	4,8-Dimethyl-3,7-nonadien-2-ol		4102 - 67845-50-5	Liquid $C_{11}H_{20}O$ 168	Insoluble Soluble	70 (2.6 hPa) n.a. - 95 %	1.465-1.473 0.860-0.870
07.099 1134	6-Methylhepta-3,5-dien-2-one		3363 11143 1604-28-0	Liquid $C_8H_{12}O$ 124.18	Almost insoluble Miscible	190 n.a. NMR 96 %	1.528-1.537 0.895-0.899
07.190 1848	Octa-1,5-dien-3-one		4405 - 65213-86-7	Liquid $C_8H_{12}O$ 124.18	Practically insoluble or insoluble Freely soluble	169 n.a. MS 95 %	1.438-1.444 0.823-0.829
07.198	Pseudo-ionone		4299 11191 141-10-6	Liquid $C_{13}H_{20}O$ 192.30	Insoluble Freely soluble	144 (16 hPa) n.a. MS 95 %	1.529-1.535 0.894-0.903
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one		- - 546-49-6	Liquid $C_{10}H_{16}O$ 152.24	Practically insoluble or insoluble Freely soluble	181 n.a. MS 95 %	1.462-1.468 0.867-0.873
07.247 1139	(E,E)-3,5-Octadien-2-one		4008 - 30086-02-3	Liquid $C_8H_{12}O$ 124.2	Insoluble Miscible	220 n.a. NMR 95 %	1.508-1.516 0.880-0.890

Table 1: Specification Summary of the Substances in the present group

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)
07.253 1139	3,5-Octadiene-2-one		- - 30086-02-3	Liquid $C_8H_{12}O$ 124.18	Freely soluble	84 (13 hPa) n.a. MS 95 %	1.509-1.515 0.867-0.873
07.256 1137	(3Z)-4,8-Dimethyl-3,7-nonadiene-2-one		3969 - 817-88-9	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Freely soluble	200-201 n.a. IR NMR 94	1.473-1.477 0.869-0.875
09.936 1847	4,8-Dimethyl-3,7-nonadien-2-yl acetate		4103 - 91418-25-6	Liquid $C_{13}H_{22}O_2$ 210	Insoluble Soluble	75-83 (3 hPa) n.a. - 95 %	1.451-1.459 0.890-0.900

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.
n.a. not applicable.

TABLE 2: CURRENT SAFETY EVALUATION STATUS APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH) (JECFA, 2009C; JECFA, 2002C)

Table 2: Summary of Safety Evaluation of the JECFA substances in the present group

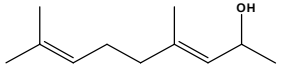
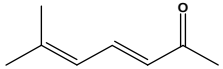
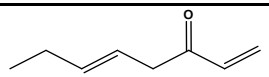
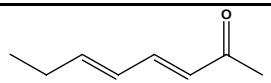
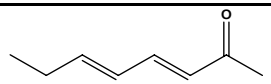
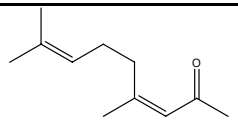
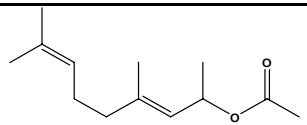
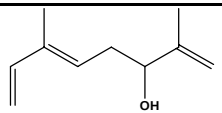
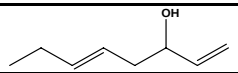
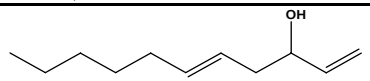
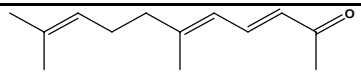
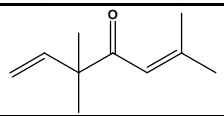
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	JECFA Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (genotoxicity)
02.252 1841	4,8-Dimethyl-3,7-nonadien-2-ol		3.0 -	Class I A3: Intake below threshold	4)	Evaluated in FGE.206, genotoxicity concern could be ruled out.
07.099 1134	6-Methylhepta-3,5-dien-2-one		13 5	Class II A3: Intake below threshold	4)	Evaluated in FGE.206, genotoxicity concern could be ruled out.
07.190 1848	Octa-1,5-dien-3-one		0.061 -	Class II A3: Intake below threshold	4)	Evaluated in FGE.206, genotoxicity concern could be ruled out.
07.247 1139	(E,E)-3,5-Octadien-2-one		3.4 4	Class II A3: Intake below threshold	4)	Evaluated in FGE.206, genotoxicity concern could be ruled out.
07.253 1139	3,5-Octadiene-2-one		0.011 3	Class II A3: Intake below threshold	4)	Evaluated in FGE.206, genotoxicity concern could be ruled out.
07.256 1137	(3Z)-4,8-Dimethyl-3,7-nonadiene-2-one		6.1 6.6	Class II A3: Intake below threshold	4)	Evaluated in FGE.206, genotoxicity concern could be ruled out.
09.936 1847	4,8-Dimethyl-3,7-nonadien-2-yl acetate		3.0 -	Class II A3: Intake below threshold	4)	Evaluated in FGE.206, genotoxicity concern could be ruled out.
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol		0.0085 -	Class II No evaluation	Not evaluated by JECFA	Evaluated in FGE.206, genotoxicity concern could be ruled out.
02.194	Octa-1,5-dien-3-ol		0.061 -	Class II No evaluation	Not evaluated by JECFA	Evaluated in FGE.206, genotoxicity concern could be ruled out.
02.211	Undeca-1,5-dien-3-ol		0.061 -	Class II No evaluation	Not evaluated by JECFA	Evaluated in FGE.206, genotoxicity concern could be ruled out.

Table 2: Summary of Safety Evaluation of the JECFA substances in the present group

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	JECFA Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (genotoxicity)
07.198	Pseudo-ionone		0.12 -	Class II No evaluation	Not evaluated by JECFA	Evaluated in FGE.206, genotoxicity concern could be ruled out.
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one		0.012 -	Class II No evaluation	Not evaluated by JECFA	Evaluated in FGE.206, genotoxicity concern could be ruled out.

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.

TABLE 3: GENOTOXICITY (*IN VITRO*)

Table 3: Summary of Additionally submitted genotoxicity data on the representative substance of subgroup 1.2.3

FL-no	Chemical Name	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
[07.099]	6-Methylhepta-3,5-dien-2-one	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	1.6, 8, 40, 200, 1000 and 5000 µg/plate [1]	Negative	(Williams, 2009)	Toxicity observed in all strains at 2000 µg/plate or greater in the absence of S9 and at 800 µg/plate in the presence of S9. Study design complied with current recommendations. Acceptable top concentration was achieved.
				20.48, 51.2, 128, 320, 800, 2000 and 5000 µg/plate [1,2]	Negative		
		Micronucleus induction	Human peripheral blood lymphocytes	225, 325 and 450 µg/ml [3] 225, 300 and 350 µg/ml [4]	Negative	(Whitwell, 2010a)	Complies with draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study.
				100, 120 or 150 µg/ml [5]	Negative		
[07.198]	Pseudo-ionone	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	0.128, 0.64, 3.2, 16, 80, 400 and 2000 µg/plate [1]	Negative	(Beevers, 2009a)	Toxicity was observed in all strains at 400 µg/plate and greater in the presence and absence of S9 in this experiment.
				0.12.5, 25, 50, 100, 200 and 400 µg/plate [1,2]	Negative		Precipitation was observed in the 400 µg/plate concentration in the presence and absence of S9 in this experiment. Study design complies with current recommendations. Acceptable top concentrations were achieved.
		Micronucleus induction	Human peripheral blood lymphocytes	30, 50 and 60 µg/ml [3] 100, 110 and 120 µg/ml [4]	Negative	(Lloyd, 2010)	Complies with draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study.
				10, 15 and 20 µg/ml [5]	Negative		

[1] With and without metabolic activation.
[2] Assay modified with pre-incubation in the presence of S9.
[3] Without metabolic activation, 3 hours treatment + 21 hours recovery.
[4] With metabolic activation, 3 hours treatment + 21 hours recovery.
[5] Without metabolic activation, 24 hours + 0 hours recovery.

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ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
ID	Identity
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	MicroNucleated BiNucleate cells
MS	Masse spectra
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear Magnetic Resonance
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
(Q)SAR	(Quantitative) Structure Activity Relationship
SCF	Scientific Committee on Food
WHO	World Health Organisation