European Food Safety Authority

EFSA Journal 2014;12(2):3587

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 210, Revision 1 (FGE.210Rev1): Consideration of genotoxic potential for α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.4 of FGE.19[1]

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

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate the genotoxic potential of 13 flavouring substances in Flavouring Group Evaluation 210 (FGE.210) and one additional substance [FL-no: 07.225] in this revision 1 (FGE.210Rev1). In the first version of FGE.210 the Panel concluded that a genotoxic potential could not be ruled out for any of the 13 substances based on data available at that time. The Flavouring Industry has now submitted additional genotoxicity data. The Panel has evaluated these data and concluded that the concern for genotoxic potential is ruled out for eight of the substances [FL-no: 02.105, 07.007, 07.009, 07.011, 07.036, 07.088, 07.091 and 07.170], while for allyl alpha-ionone [FL-no: 07.061] and for alpha-damascone [FL-no: 07.134] and four structurally related substances [FL-no: 07.130, 07.225, 07.226 and 07.231] the concern still remains with respect to genotoxicity.

KEY WORDS

α,β-unsaturated ketones, flavouring substances, safety evaluation, FGE.210, subgroup 2.4, FGE.19

[1] On request from the European Commission, Question No EFSA-Q-2013-00268 to EFSA-Q-2013-00276 and EFSA-Q-2013-00555 to EFSA-Q-2013-00559, adopted on 30 January 2014.

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Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 210 Revision 1 (FGE.210Rev1): Consideration of genotoxic potential for α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.4 of FGE.19. EFSA Journal 2014;12(2):3587, 35 pp. doi:10.2903/j.efsa.2014.3587

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000.

The Flavouring Group Evaluation 210 (FGE.210) concerned 13 substances, belonging to subgroup 2.4 of FGE.19. Twelve of these substances are α,β-unsaturated alicyclic ketones (alpha-ionone [FL-no: 07.007], methyl-alpha-ionone [FL-no: 07.009], 4-(2,5,6,6-tetramethyl-2-cyclohexenyl)-3-buten-2-one [FL-no: 07.011], alpha-isomethyl ionone [FL-no: 07.036], allyl alpha-ionone [FL-no: 07.061], methyl-delta-ionone [FL-no: 07.088], gamma-ionone [FL-no: 07.091], delta-damascone [FL-no: 07.130], alpha-damascone [FL-no: 07.134], beta-ionone epoxide [FL-no: 07.170], tr-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)but-2-en-1-one [FL-no: 07.226] and alpha-damascenone [FL-no: 07.231]) and one substance (4-(2,6,6-trimethyl-2-cyclohexenyl)but-3-en-2-ol [FL-no: 02.105]) is a precursor for such ketone. One of the substances (allyl alpha-ionone [FL-no: 07.061]) has a terminal double bond and one (beta-ionone epoxide [FL-no: 07.170]) is an epoxide .

The genotoxicity concern with respect to the 13 α,β-unsaturated alicyclic ketones and precursors in FGE.210 could not be ruled out based on the genotoxicity data and (Quantitative) Structure-Activity Relationship ((Q)SAR) predictions available.

The present revision of FGE.210 (FGE.210Revision1) has been prepared due to additional genotoxicity data submitted by the Industry on representative substances (alpha-ionone [FL-no: 07.007], allyl alpha-ionone [FL-no: 07.061], beta-ionone epoxide [FL-no: 07.170] and alpha-damascone [FL-no: 07.134]). Also an additional substance has been included, cis-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)but-2-en-1-one [FL-no: 07.225].

The Panel has evaluated these data and concluded that the concern for genotoxic potential is ruled out for eight of the substances [FL-no: 02.105, 07.007, 07.009, 07.011, 07.036, 07.088, 07.091 and 07.170]. These eight substances can accordingly be evaluated using the Procedure. For allyl alpha-ionone [FL-no: 07.061] and for alpha-damascone [FL-no: 07.134] and the four structurally related substances [FL-no: 07.130, 07.225, 07.226 and 07.231], the new submitted data could not rule out the Panel concern with respect to genotoxicity and additional data are requested.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[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 13 flavouring substances, which correspond to subgroup 2.4 of FGE.19, in its evaluation of the flavouring group 210 (FGE.210). The opinion was adopted on 29 January 2009.

EFSA concluded that a genotoxic potential of these α,β-unsaturated alicyclic ketones and precursors in the present FGE.210 could not be ruled out.

Information on three representative materials alpha-ionone [FL-no: 07.007], allyl alpha-ionone [FL-no: 07.061] and beta-ionone epoxide [FL-no: 07.170] has now been submitted by the European Flavour Association. This information is intended to cover the re-evaluation of the above mentioned substances and of six substances from FGE.19 subgroup 2.4 [FL-no: 02.105, 07.009, 07.011, 07.036, 07.088 and 07.091].

- 4-(2,6,6-Trimethyl-2-cyclohexenyl)but-3-en-2-ol [FL-no: 02.105]
- Methyl-alpha-ionone [FL-no: 07.009]
- 4-(2,5,6,6-Tetramethyl-2-cyclohexenyl)-3-buten-2-one [FL-no: 07.011]
- alpha-Isomethyl ionone [FL-no: 07.036]
- Methyl-delta-ionone [FL-no: 07.088]
- gamma-Ionone [FL-no: 07.091]

Additionally, new information on one further representative material alpha-damascone [FL-no: 07.134] and on one other substance, delta-damascone [FL-no: 07.130], has now been submitted by the European Flavour Association. This information is intended to cover also the re-evaluation of the following flavouring substances from FGE.19 subgroup 2.4

- cis-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)but-2-en-1-one [FL-no: 07.225]
- tr-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)but-2-en-1-one [FL-no: 07.226]
- alpha-damascenone [FL-no: 07.231]

[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 (EC) 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.

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 flavouring substances in the present FGE: 4-(2,6,6-trimethyl-2-cyclohexenyl)but-3-en-2-ol [FL-no: 02.105], alpha-ionone [FL-no: 07.007], methyl-alpha-ionone [FL-no: 07.009], 4-(2,5,6,6-tetramethyl-2-cyclohexenyl)-3-buten-2-one [FL-no: 07.011], alpha-isomethyl ionone [FL-no: 07.036], allyl alpha-ionone [FL-no: 07.061], methyl-delta-ionone [FL-no: 07.088], gamma-ionone [FL-no: 07.091], delta-damascone [FL-no: 07.130], alpha-damascone [FL-no: 07.134], beta-ionone epoxide [FL-no: 07.170], cis-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)but-2-en-1-one [FL-no:07.225], tr-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)but-2-en-1-one [FL-no:07.226] and alpha-damascenone [FL-no: 07.231] in accordance with Commission Regulation (EC) No 1565/2000.

HISTORY OF THE EVALUATION OF FGE.19 SUBSTANCES

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

The α,β-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity (EFSA, 2008a). 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 α,β-unsaturated carbonyls were subdivided into subgroups on the basis of structural similarity (EFSA, 2008a). 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 and Netzeva, 2007a; Benigni and 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, 2008a) 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 ease the data retrieval of the large number of structurally related α,β-unsaturated substances in the different subgroups for which additional data are requested, EFSA worked out a list of representative substances for each subgroup (EFSA, 2008c). Likewise an EFSA genotoxicity expert group has worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008b).

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.

ASSESSMENT

1. History of the Evaluation of the Substances Belonging to FGE.210

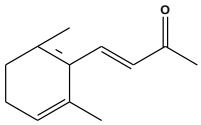
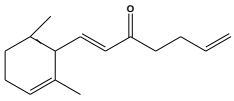
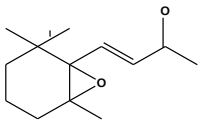
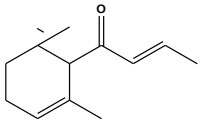
In FGE.210 EFSA considered 13 flavouring substances corresponding to subgroup 2.4 of FGE.19. Twelve of these substances are α,β-unsaturated alicyclic ketones [FL-no: 07.007, 07.009, 07.011, 07.036, 07.061, 07.088, 07.091, 07.130, 07.134, 07.170, 07.226 and 07.231] and one (4-(2,6,6-trimethyl-2-cyclohexenyl)but-3-en-2-ol [FL-no: 02.105]) is a precursor for such ketone. One of the substances (allyl alpha-ionone [FL-no: 07.061]) has a terminal double bond and one (beta-ionone epoxide [FL-no: 07.170]) is an epoxide.

The genotoxicity concern with respect to the 13 α,β-unsaturated alicyclic ketones and precursors could not be ruled out based on the genotoxicity data and the (quantitative) structure-activity relationship ((Q)SAR) predictions available. The Panel therefore concluded that additional data on genotoxicity on substances representative for this subgroup should be provided according to the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19 (EFSA, 2008b).

FGE	Adopted by EFSA	Link	No. of Substances
FGE.210	29 January 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1030.htm	13
FGE.210Rev1	30 January 2014		14

The present revision of FGE.210 (FGE.210Rev1) concerns the evaluation of additional data submitted by Industry for the representative substances alpha-ionone [FL-no: 07.007], allyl alpha-ionone [FL-no: 07.061], beta-ionone epoxide [FL-no: 07.170] (IOFI, 2013a) and alpha-damascone [FL-no: 07.134] (IOFI, 2013b) (Table 1). Data on beta-damascone [FL-no: 07.083] (belonging to subgroup 2.7b of FGE.19) have also been provided as representative of the damascone-type substances. However, the Panel disagrees with this structural related similarity and beta-damascone [FL-no: 07.083] will be considered a representative substance only for subgroup 2.7b of FGE.19 (FGE.213) as indicated in the "List of alpha- and beta-unsaturated aldehydes and ketone representative of FGE.19 substances for genotoxicity" (EFSA, 2008c). Furthermore, data on genotoxicity of delta-damascone [FL-no: 07.130] have been submitted.

Table 1: Representative substances for Subgroup 2.4 of FGE.19 (EFSA, 2008c)

Subgroup	FL-no	Register name for representatives	Structural formula
Ionone-related	07.007	alpha-Ionone	
	07.061	Allyl alpha-ionone	
	07.170	beta-Ionone epoxide	
Damascone-related	07.134	alpha-Damascone	

Since the previous version of FGE.210 one additional substance has been included in subgroup 2.4, cis-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)but-2-en-1-one [FL-no: 07.225].

Section 2 and 3 report the same information that was presented in the previous version of FGE.210. Section 4 report the new submitted data.

2. Presentation of the Substances in Flavouring Group Evaluation 210Rev1

2.1. Description

The Flavouring Group Evaluation 210 (FGE.210) concerns 14 substances, corresponding to subgroup 2.4 of FGE.19 (see Table 2). Thirteen of these substances are α,β-unsaturated alicyclic ketones [FL-no: 07.007, 07.009, 07.011, 07.036, 07.061, 07.088, 07.091, 07.130, 07.134, 07.170, 07.225 (not present in FGE.210), 07.226 and 07.231] and one is a precursor for such ketone [FL-no: 02.105]. One of the substances has a terminal double bond [FL-no: 07.061] and one is an epoxide [FL-no: 07.170].

Eleven of the substances in the present FGE have previously been evaluated by the JECFA, a summary of their current evaluation status by the JECFA is given in Table 3 (JECFA, 1999; JECFA, 2006).

As the α,β-unsaturated ketone structure is a structural alert for genotoxicity (EFSA, 2008a), the available data on genotoxic or carcinogenic activity for the 13 α,β-unsaturated ketones [FL-no: 07.007, 07.009, 07.011, 07.036, 07.061, 07.088, 07.091, 07.130, 07.134, 07.170, 07.225, 07.226 and 07.231] and a precursor for such ketone [FL-no: 02.105], will be considered in this FGE.

3. Data evaluated by the Panel in FGE.210[7]

3.1. (Q)SAR Predictions

The Panel has also taken into consideration the outcome of the predictions from five selected (Quantitative) Structure-Activity Relationship ((Q)SAR) models (Benigni & Netzeva, 2007a; Gry et al., 2007; Nikolov et al., 2007) on twelve ketones [FL-no: 07.007, 07.009, 07.011, 07.036, 07.061, 07.088, 07.091, 07.130, 07.134, 07.170, 07.226 and 07.231] in the original version of FGE.210.

In Table 4 the outcomes of the (Q)SAR predictions for possible genotoxic activity in five *in vitro* (Q)SAR models (ISS-Local Model-Ames test, DTU-NFI MultiCASE-Ames test, Chromosomal aberration test in Chinese hamster ovary (CHO) cells, Chromosomal aberration test in Chinese hamster lung (CHL) cells, and Mouse lymphoma test) are presented.

For all of the substances the (Q)SAR models predict negative results in tests for gene mutations, with the restriction that about half of the substance predictions are out of domain for the Mouse lymphoma assay. It is noted that predictions for chromosomal aberrations (CA) are diverging in the sense that for CAs in Chinese hamster ovary cells the predictions are invariably negative (three are out of domain), while for the same endpoint in another but very similar cell type (Chinese hamster lung cells) only for one substance a negative response was predicted. For most of the remaining substances the predictions in the CA (CHL) test were equivocal and for four substances the predictions were out of domain (Table 4).

[7] The data presented in Section 3 is cited from the first version of FGE.210. These data are the basis for the conclusions in FGE.210 requesting additional genotoxicity data.

3.2. Genotoxicity Studies

In subgroup 2.4 there are two *in vitro* studies on alpha-ionone [FL-no: 07.007], one *in vitro* study on methyl-alpha-ionone [FL-no: 07.009] and one *in vitro* study on methyl-delta-ionone [FL-no: 07.088]. Only one *in vivo* study for methyl-alpha-ionone [FL-no: 07.009] is available for this subgroup.

Study validation and results are presented in Tables 5 and 6.

The available *in vitro* bacterial gene mutation studies with limited validities do not indicate a concern for the tested substances from this group. One of the *in vitro* tests (Rec assay) is in a system which has limited predictive validity for genotoxicity. An *in vivo* test with limited validity produced a negative result for gene mutations in *Drosophila melanogaster*. A limited *in vitro* test for structural chromosomal damage produced a positive response with alpha-ionone, but a limited *in vivo* mammalian test for the same endpoint with alpha-ionone gave a negative outcome.

3.3. Carcinogenicity Studies

No carcinogenicity studies are available for the substances in subgroup 2.4.

3.4. Conclusion on Genotoxicity and Carcinogenicity

The data ((Q)SAR and testing data) are not sufficient to rule out a concern for genotoxicity for these substances.

3.5. Conclusions

The Panel concluded that a genotoxic potential of the 13 α,β-unsaturated alicyclic ketones and precursors in the present FGE.210 [FL-no: 02.105, 07.007, 07.009, 07.011, 07.036, 07.061, 07.088, 07.091, 07.130, 07.134, 07.170, 07.226 and 07.231] could not be ruled out based on the data available. Accordingly these 13 substances cannot be evaluated through the Procedure, presently. Additional data on genotoxicity on substances representative for this subgroup should be provided according to the Genotoxicity Test Strategy for substances belonging to Subgroups of FGE.19 (EFSA, 2008b).

4. Additional Genotoxicity Data Submitted for FGE.19 Subgroup 2.4 (FGE.210)

4.1. Presentation of the Additional Data

The present revision of FGE.210 (FGE.210Rev1), concerns the evaluation of additional data submitted by Industry for the representative substances alpha-ionone [FL-no: 07.007], allyl alpha-ionone [FL-no: 07.061], beta-ionone epoxide [FL-no: 07.170] and alpha-damascone [FL-no: 07.134] for subgroup 2.4 (EFSA, 2008a). Furthermore, data on genotoxicity of delta-damascone [FL-no: 07.130] have been submitted.

In response to the EFSA request in FGE.210 for additional genotoxicity data for subgroup 2.4, the Flavour Industry (IOFI, 2013a; 2013b) has submitted genotoxicity data on:

Substance	**Bacterial Mutation**	***In vitro* Micronucleus**	***In vivo* Micronucleus**
alpha-Ionone [FL-no: 07.007]	(Bowen, 2011)	(Lloyd, 2013b)	(Krsmanovic and Huston, 2006)
Allyl alpha-ionone [FL-no: 07.061]	(Ballantyne, 2011)	(Lloyd, 2013a)	
delta-Damascone [FL-no: 07.130]	(Shinya, 2006)		
alpha-Damascone [FL-no: 07.134]	(Haddouk, 2001)	(Lloyd, 2012; Lloyd, 2013c; Whitwell, 2012)	
beta-Ionone epoxide [FL-no: 07.170]	(Jones and Wilson, 1988; Kringstad, 2005)	(Flanders, 2006)	

4.2. *In vitro* data

4.2.1. Bacterial Reverse Mutation Assay

alpha-Ionone [FL-no: 07.007]

An Ames assay was conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 to assess the mutagenicity of alpha-ionone, both in the absence and in the presence of metabolic activation by S9-mix (from livers of rats induced with Aroclor 1254), in three separate experiments (Bowen, 2011). This study was performed following GLP recommendations and according to OECD Guideline 471 (OECD, 1997a). An initial experiment was carried out both in the absence and presence of S9-mix activation in all five strains, using 0.3, 1.6, 8, 40, 200, 1000 and 5000 µg of alpha-ionone/plate, plus negative (solvent) and positive controls. Evidence of toxicity was observed at 1000 and/or 5000 µg/plate across all strains in the absence and presence of S9-mix with the exception of TA100 in which no clear evidence of toxicity, in presence of S9-mix, was observed at 5000 µg/plate (Table 7).

In a second experiment, the concentrations were changed lowering to 2500 µg/plate for all strains and conditions with the exception of TA98 in presence of S9-mix and for TA100 in presence and absence of S9-mix. In this second experiment the concentration intervals were narrowed, covering the ranges 156.3 - 5000 µg/plate or 78.1 - 2500 µg/plate in order to better detect possible concentration-dependent mutation. In addition, a pre-incubation step with S9-mix activation treatment was added to increase the chance of detecting a positive response. In this experiment, evidence of toxicity ranging from a diminution of the background bacterial lawn and/or a reduction in revertant numbers to a complete killing of the test bacteria was observed at 1250 µg/plate and above for strain TA98 in the presence of S9-mix, at 625 µg/plate in strains TA98 in the absence of S9-mix and TA100 with and without S9-mix. Toxicity was observed at 312.5 µg/plate and above in all remaining strains.

The third experiment was conducted using strains TA1535 and TA102 in the absence and presence of S9-mix activation and strain TA1537 in the presence of S9-mix activation. In the treatment with S9-mix a pre-incubation step was included. The maximum test concentration was 2500 µg/plate for TA1535 while it was further reduced for TA102 (with or without S9-mix) and for TA1537 to 1250 µg/plate. In addition, more narrow concentration intervals were used, covering either 39.1 to 2500 µg/plate or 19.5 to 1250 µg/plate. Evidence of toxicity was observed at the highest three or four concentrations across all strains in the absence or presence of S9-mix.

In all three experiments, 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.

It was concluded that alpha-ionone did not induce mutations in five strains of *S. typhimurium*, when tested under the conditions of this study.

Allyl alpha-ionone [FL-no: 07.061]

An Ames assay was conducted in *S. typhimurium* strain TA102 to assess the mutagenicity of allyl alpha-ionone, both in the absence and in the presence of metabolic activation by S9-mix (from livers of rats induced with Aroclor 1254), in two separate experiments (Ballantyne, 2011). The study was performed following GLP principles and according to the OECD Guideline 471 (OECD, 1997a), except that only TA102 was used (Table 7). An initial experiment was carried out both in the absence and presence of S9-mix activation in the TA102 strain, using 1.6, 8, 40, 200, 1000 and 5000 µg of allyl alpha-ionone/plate plus vehicle and positive controls. In the second experiment, the highest concentration was retained but more narrow concentration intervals were used, starting at 51.2 µg/plate (51.2, 128, 320, 800, 2000 and 5000 µg/plate). The standard plate incorporation assay was used in the first experiment and a pre-incubation step with S9-mix activation treatment was added in

the second experiment to increase the chance of detecting a positive response. No evidence of toxicity was observed under any of the conditions tested.

In both experiments, no statistically significant increases in revertant numbers were observed at any concentration in strain TA102, either in the presence or absence of S9-mix activation.

It was concluded that allyl alpha-ionone did not induce mutation in the histidine-requiring *S. typhimurium* strain TA102 when tested under the conditions of this study. The authors justified to test only TA102 strain because this study was intended to be complementary to a previous study from (Wild et al., 1983) where data on the other strains were provided.

delta-Damascone [FL-no: 07.130]

A modified Ames assay using the pre-incubation method was conducted in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2uvrA to assess the mutagenicity of delta-damascone (purity: 93.8 %), both in the absence and in the presence of metabolic activation by S9-mix (from livers of rats induced with Aroclor 1254), in three separate experiments (Shinya, 2006). The assay was performed according to OECD Guideline 471 (OECD, 1997a) and according to GLP principles (Table 7).

An initial experiment was carried out both in the absence and presence of S-9 mix activation in all five strains at 4.9, 19.5, 78.1, 313, 1250 and 5000 µg of delta-damascone/plate), plus negative (solvent) and positive controls. In the absence of S9-mix, toxicity (decrease of bacterial growth and/or of revertants) was reported at 78.1 µg/plate and above and in the presence of S9-mix, toxicity was reported at 313 µg/plate and above. In the second experiment with tighter ranges of concentrations to reflect the toxicity observed in the previous experiment, delta-damascone was incubated with all five tester strains in the absence of S9-mix (2.4, 4.9, 9.8, 19.5, 39.1 or 78.1 µg of delta-damascone/plate) and in the presence of S9-mix (9.8, 19.5, 39.1, 78.1, 156 and 313 µg of delta-damascone/plate). Toxicity was observed in the absence of S-9 mix at top concentration and in the presence of S9-mix at 156 µg/plate and above. In the third experiment, the same conditions as described for the second experiment were used. In all three experiments there were no significant increases in the number of revertants in the absence or presence of S9-mix. It was concluded that delta-damascone did not induce mutations in four strains of *S. typhimurium* or *E. coli* WP2uvrA under the conditions employed (Shinya, 2006).

alpha-Damascone [FL-no: 07.134]

Ames assays were conducted in *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2uvrA to assess the mutagenicity of alpha-damascone (purity: 96.9 %), both in the absence and in the presence of metabolic activation by S9-mix (from livers of rats induced with Aroclor 1254), in two separate experiments (Haddouk, 2001). The assay was performed according to OECD Guideline 471 (OECD, 1997a) and according to GLP principles (Table 7).

An initial experiment to assess toxicity was carried out both in the absence and presence of S9-mix activation in the tester strains, using 10, 100, 500, 1000, 2500 and 5000 µg of alpha-damascone/plate in strains TA98, TA100 and WP2 uvrA, plus vehicle and positive controls. Concentration levels greater than or equal to 2500 µg/plate showed evidence of an emulsion on the plates. In TA98, slight to marked toxicity was observed at concentrations greater than or equal to 100 µg/plate or 500 µg/plate in the absence and presence of S9-mix, respectively. In TA100, toxicity was observed at concentrations greater than or equal to 500 µg/plate in the absence and presence of S9-mix. In *E. coli* WP2 uvrA slight toxicity was observed at 2500 µg/plate and above without S9-mix but not with S9-mix. Based on the preliminary toxicity test, a standard Ames test using the plate incorporation method was conducted using 31.2, 62.5, 125, 250 and 500 µg of alpha-damascone/plate for strains TA1535 and TA100 and 7.8, 15.6, 31.2, 62.5 and 125 µg of alpha-damascone/plate for strains TA1537 and TA98 in the absence and presence of S9-mix. Additionally alpha-damascone (312.5, 625, 1250, 2500 and 5000 µg/plate) was tested in *E. coli* WP2 uvrA for reverse mutation in the absence and presence of

S9-mix. In the second experiment, alpha-damascone was tested in all *S. typhimurium* tester strains in the absence of S9-mix at the following concentrations: 15.6, 31.2, 62.5, 125 and 250 µg/plate. In the second experiment, the tests run in the presence of S9-mix were performed with the pre-incubation (modified Ames) method at concentrations of 31.2, 62.5, 125, 250 and 500 µg of alpha-damascone/plate for strains TA1535 and TA100 and at concentrations of 15.6, 31.2, 62.5, 125 and 250 µg of alpha-damascone/plate for strains TA1537 and TA98. Additionally alpha-damascone (312.5, 625, 1250, 2500 and 5000 µg/plate) was tested in *E. coli* WP2 uvrA for reverse mutation in the absence of S9-mix (with the plate incorporation method) and in the presence of S9-mix (with the pre-incubation method). Slight evidence of toxicity was observed under the conditions tested through thinning of the background bacterial lawn and/or a decrease in revertant count in *Salmonella* strains. In the *E. coli* strain, a slight toxicity was observed only at 5000 µg/plate in the absence of S9-mix.

In both experiments, 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.

It was concluded that alpha-damascone did not show mutagenic activity towards *S. typhimurium* or *E. coli* in the bacterial reverse mutation test (Haddouk, 2001). The Panel agreed with the conclusion of the author.

beta-Ionone epoxide [FL-no: 07.170]

beta-Ionone epoxide was tested for mutagenicity in an Ames test including four strains of *S. typhimurium* (TA98, TA100, TA1535 and TA1537) at five concentrations (5, 15, 50, 150, 500 µg/plate) in the absence and in the presence of metabolic activation (S9-mix at two different concentrations, 3 and 10 %) (Jones and Wilson, 1988). The study was performed under GLP and mainly compliant with OECD Guideline 471 (OECD, 1997a), except that only four strains were used (Table 7). Two independent experiments were performed and the top concentration was selected at 500 µg/plate based on toxicity in a prior range-finding test. At the concentration tested no significant toxicity was observed and no substantial increases in mutation were observed in all strains tested and in presence or absence of S9.

A more recently reported Ames study on beta-ionone-epoxide included four strains of *S. typhimurium* (TA97a, TA98, TA100, TA1535) plus one strain of *E. coli* (WP2-uvrA-) (Kringstad, 2005). Following a range-finding assay, beta-ionone was tested in three replicates at 501, 1582 and 5000 µg/plate in the absence of S9-mix metabolic activation and at 158, 501 and 1582 µg/plate in the presence of metabolic activation, in a single experiment using the plate incorporation method. The top concentration (5000 µg/plate) induced significant toxicity in strain TA97a in the absence of S9-mix and also reduced the background lawn in strain TA100 in the presence and absence of S9-mix and therefore the study complies with current recommendations for the choice of concentration. There was no evidence of mutagenicity. Since there are some deviations from the OECD Guideline 471 (OECD, 1997a) (only three concentrations of chemical were tested, in some cases only two concentrations could be analysed due to an excessive level of cytotoxicity and only a single experiment was performed) the test is considered of limited validity.

4.2.2. Mouse lymphoma thymidine kinase gene mutation assay

beta-Ionone epoxide [FL-no: 07.170]

An assay for induction of *tk* mutations in mouse lymphoma cells (L5178Y T/K +/- 3.7.2c) was conducted on beta-ionone-epoxide (Flanders, 2006). It included 4 hours treatment in the absence and presence of S9-mix and a 24 hours treatment in the absence of S9-mix. The concentrations were selected based on a preliminary toxicity test. The test groups included single replicates at 8 concentrations ranging from 200 to 900 µg/ml in the 4 hours treatment arm and from 4.1 to 520 µg/ml in the 24 hours treatment arm. The maximum concentration was limited by toxicity. The substance did not induce biologically or statistically significant increases in mutant frequency and therefore it was

considered non-mutagenic in this assay. The study is compliant with OECD Guideline 476 (OECD, 1997c) (Table 7).

4.2.3. Micronucleus Assays

alpha-Ionone [FL-no: 07.007]

alpha-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-mix fraction (Table 7). Information about the method used to induce lymphocyte cell division and the duration of the induction before the treatment was not provided. Cells were treated for 24 hours with 14 concentrations in a range from 15 to 120 µg/ml of alpha-ionone in absence of S9-mix. In presence of S9-mix, cells were exposed for 3 hours followed by 21 hours recovery with 15 concentrations in a range from 30 to 200 µg/ml. Based on the toxicity induced by alpha-ionone, three concentrations were selected for micronuclei assessment. In absence of S9-mix, cells were treated with 40, 50 and 65 µg/mL, while in presence of S9-mix the concentrations selected were 160, 170 and 180 µg/ml. The highest concentrations induced 51 % and 56 % reduction of replicative index (RI) in absence and presence of S9-mix, respectively. Micronuclei assessment was performed in a single experiment with duplicates and a total of 1000 binucleate cells per replicate were scored. No assay with 3-hour treatment + 21-hour recovery in absence of S9-mix was performed as recommended by OECD Guideline 487 (OECD, 2010). Treatment of cells with alpha-ionone for 3 hours with a 21 hours recovery period in the presence of S9-mix or for 24 hours with no recovery period in the absence of S9-mix showed no increase in the frequency of MNBN cells at any concentration when compared to both concurrent and historical controls. It was concluded that alpha-ionone did not induce micronuclei at up to the limit of toxicity when assayed in cultured human peripheral lymphocytes under the described exposure conditions (Lloyd, 2013b).

Due to the deviation from the OECD Guideline 487 (OECD, 2010) the study is considered of limited validity.

Allyl alpha-ionone [FL-no: 07.061]

Allyl alpha-ionone was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes (Table 7). Information about the method used to induce lymphocytes cell division and the duration of the induction before the treatment was not provided. Cells were treated for 24 hours with 14 concentrations in a range from 5 to 50 µg/ml of allyl alpha-ionone in absence of S9-mix. In presence of S9-mix cells were exposed for 3 hours followed by 21 hours recovery with 15 concentrations in a range from 25 to 200 µg/ml. Based on the toxicity induced by alpha-ionone three concentrations were selected for micronuclei assessment. In absence of S9-mix (24 hours treatment), cells were treated with 25, 33, 36 and 38 µg/ml, while in presence of S9-mix (3 hours treatment) the concentrations selected were 110, 140, 150 and 160 µg/ml. The highest concentrations induced 54 % and 63 % reduction of RI in absence and presence of S9-mix, respectively. Micronuclei assessment was performed in a single experiment with duplicates and a total of 2000 binucleate cells per replicate were scored in the experiment performed in absence of S9-mix, while 1000 binucleated cells were scored in presence of S9-mix. No assay with 3-hour treatment + 21-hour recovery in absence of S9-mix was performed as recommended by OECD Guideline 487 (OECD, 2010).

Treatment of cells with allyl alpha-ionone for 3 hours with a 21 hours recovery period in the presence of S9-mix or for 24 hours with no recovery period in the absence of S9-mix showed no increase in the frequency of MNBN cells at any concentration when compared to both concurrent and historical controls. It was concluded that allyl alpha ionone did not induce micronuclei at concentration up to the limit of toxicity when assayed in cultured human peripheral lymphocytes in the described exposure conditions (Lloyd, 2013a).

Due to the deviation from the OECD Guideline 487 (OECD, 2010) the study is considered of limited validity.

alpha-Damascone [FL-no: 07.134]

Three *in vitro* micronucleus experiments have been performed in human peripheral blood lymphocytes to determine whether alpha-damascone is able to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system (Table 7).

In all three experiments cells were stimulated for 48 hours with phytohaemaglutinin to produce exponentially growing cells.

A first experiment (Lloyd, 2012) was performed using standard conditions. Human peripheral blood lymphocytes were treated with alpha-damascone (purity 98.3 %) for 3 hours (followed by 21 hours recovery) with 9, 16, 18 or 22 µg/ml and 12, 18, 20, 21 or 22 µg/ml of alpha-damascone in the absence and presence of S9-mix, respectively. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 55 % and 56 %, respectively. In a parallel assay, cells were treated for 24 hours with 5, 7, 9 and 10 µg/ml of alpha-damascone in the absence of S9-mix with no recovery period. The top concentration induced 57 % cytotoxicity. Cytotoxicity was observed at the top concentrations in all parts of the study. There were two replicate cultures per treatment and 1000 binucleate cells per replicate were scored for micronuclei. The study design complies with OECD Guideline 487 (OECD, 2010) and follows GLP principles. Treatment of cells with alpha-damascone for 3 hours with a 21 hour recovery period in the absence of S9-mix or for 24 hours with no recovery period in the absence of S9-mix showed no increase in the frequency of MNBN cells at any concentration when compared to both concurrent and historical controls. Treatment of cells with alpha-damascone for 3 + 21 hours in the presence of S9-mix resulted in frequencies of MNBN cells that were significantly higher ($p < 0.001$) when compared to concurrent controls at the two highest test concentrations with 1.40 and 1.70 % MNBN at 21 and 22 µg/ml compared to 0.25 % MNBN cells in the concurrent control. It is noted that although the frequencies of MNBN cells exceed the 95th percentile of the historical controls (0.1 - 1.2 % MNBN) they are still within the normal range when considering the total range (0 - 2.0 % MNBN). An additional reading on 1000 BN cells scored per replicate confirmed the statistically significant increase with 1.03 and 1.0 % MNBN cells at 21 and 22 µg/ml; however, no additional reading was performed in the controls. It was concluded that alpha-damascone showed weak induction of micronuclei when assayed in cultured human peripheral lymphocytes for 3+21 hours in the presence of S9-mix, while in absence of S9-mix no induction of micronuclei were observed when tested up to toxic concentrations for 3 + 21 hours and 24 + 0 hours (Lloyd, 2012).

Since this study (Lloyd, 2012) showed a variable toxicity profile in the treatment for 3 hours with a 21 hours recovery period in the presence of S9-mix, a follow-up *in vitro* micronucleus assay was performed (Whitwell, 2012). alpha-Damascone was tested on human lymphocyte cultures using different methods of addition/mixing the test substance to the treatment medium, in order to assess and compare the cytotoxicity. A high variability in cytotoxicity was observed and it was concluded that where alpha-damascone is prepared for 100 % medium replacement and treated in a large volume vessel with vigorous mixing, a smoother and steeper toxicity curve is obtained as compared to using a standard method of addition to the test system. The micronucleus assay was performed using 3 different methods of adding/mixing alpha-damascone to the treatment medium: standard treatment in larger volume vessel (experiment 1), standard treatment in standard vessel (experiment 2) and 100 % medium replacement in a larger volume vessel (experiment 3). The following concentrations were tested: 7.5 to 14 µg/ml (experiment 1 and 3), 14 to 20 µg/ml (experiment 2). Data indicated a positive induction of MNBN cells for at least one concentration for each experiment with a concentration-dependent effect.

Repetition of the experiments under slightly different conditions (with respect to the volume of vessel, mixing conditions and medium replacement) resulted in similar induction of micronuclei. The author of the repeated study concluded that alpha-damascone did not induce consistent and biologically

relevant increases in the frequency of micronuclei in cultured human peripheral blood lymphocytes when tested for 3+21 hours in the presence of S9-mix and for 24+0 hours in the absence of S9-mix (Lloyd, 2013c).

The Panel noted that statistically significant increases of MNBN cells were observed at concentrations that are above the limits of cytotoxicity recommended by the guideline and that the increases were higher than the 95th percentile of the historical control, but that the effects were observed only at the high concentrations at a cytotoxicity level higher than 55 %. Under these conditions, the Panel concluded that alpha-damascone presents, in this study, an equivocal effect in the *in vitro* micronucleus test.

The results of *in vitro* micronucleus studies are summarised in Table 7.

4.3. *In vivo* data

4.3.1. Bone Marrow Micronucleus Assay

alpha-Ionone [FL-no: 07.007]

alpha-Ionone was tested in a mouse bone marrow micronucleus assay (Krsmanovic and Huston, 2006). An initial extensive range-finding test established a maximum tolerated dose (MTD) of 1200 mg/kg. Animals were dosed by a single intraperitoneal injection, either with vehicle or with alpha-ionone at 300, 600 or 1200 mg/kg. Groups of 5 male and 5 female mice from all treatment levels were sacrificed 24 hours after dosing, and additional 5 mice of each sex from top dose and vehicle control groups were also sacrificed at 48 hours after dosing (Table 8).

2000 Polychromatic (PCE) and normochromatic (NCE) erythrocytes per animal were scored for micronuclei. Reductions of 7 - 18 % in PCE were observed in treated males and females at 24 hours after dosing; a reduction of 21 % in PCE was observed in males at the top dose at 48 hours after dosing, indicating bone marrow toxicity. Systemic availability was confirmed by additional clinical signs in treated animals. There were no statistically or biologically significant increases in micronucleus frequency in treated animals. The study is compliant with OECD Guideline 474 (OECD, 1997b) and is sufficiently robust to contribute to the evaluation of clastogenic or aneugenic potential of alpha-ionone.

CONCLUSION

In the first evaluation of the available data on α,β-unsaturated alicyclic ketones and precursors in FGE.210 (subgroup 2.4 of FGE.19), it was concluded that additional data should be provided for the proper consideration of the genotoxic potential of these substances (EFSA, 2009).

New *in vitro* data have been submitted for five substances of FGE.19 subgroup 2.4 (FGE.210), four representatives (alpha-ionone [FL-no: 07.007], alpha-damascone [FL-no: 07.134], allyl alpha-ionone [FL-no: 07.061], beta-ionone epoxide [FL-no: 07.170]), as requested, and one other substance, delta-damascone [FL-no: 07.130]. Furthermore, new *in vivo* data have been submitted for the representative alpha-ionone [FL- no: 07.007].

alpha-Ionone [FL-no: 07.007] did not induce gene mutation in *S. typhimurium* nor structural or numerical chromosomal aberrations when tested with human peripheral lymphocytes. The latter study is of limited validity due to deviation to the OECD Guideline 487 (OECD, 2010) and it is not a GLP study. However, alpha-ionone was tested in an *in vivo* mouse bone marrow micronucleus assay in which no statistically significant increase in the frequency of micronucleated cells was observed. There was an indication for bone marrow exposure, thus, the result is considered reliable.

The new data submitted for beta-ionone epoxide [FL-no: 07.170] included two *in vitro* studies in bacteria and mammalian cells. beta-Ionone epoxide did not induce any significant increase in bacterial

mutation when evaluated in five different *S. typhimurium* strains and an *E. coli* strain, either in the presence or absence of S9 metabolic activation in two independent studies. beta-Ionone epoxide also did not increase mutation frequencies when tested in a *tk* mutation assay using mouse lymphoma cells either in the presence or absence of S9 metabolic activation. No *in vitro* assay for chromosomal aberration is available, but the mouse lymphoma assay is a test that is able to detect the chemical potential to induce structural chromosomal aberrations. The lack of an *in vitro* micronucleus assay is not consistent with the current EFSA guideline (EFSA Scientific Committee, 2011), it is however consistent with the genotoxicity test strategy for substances belonging to subgroups of FGE.19 (EFSA, 2008b) applicable at the time when the scientific opinion on FGE.210 was adopted (EFSA, 2009). The Panel concluded that the data submitted for beta-ionone epoxide are sufficient in the light of data available for structurally related substances.

Therefore, the Panel concluded that based on the current data on the representative substances alpha-ionone [FL-no: 07.007] and beta-ionone epoxide [FL-no: 07.170], the concern with respect to genotoxicity could be ruled out as well as for the six other substances [FL-no: 02.105, 07.009, 07.011, 07.036, 07.088 and 07.091] structurally related to ionone. Accordingly these eight substances can be evaluated through the procedure.

Allyl alpha-ionone [FL-no: 07.061] is, due to a terminal double bond, not considered sufficiently structurally related to the other ionones. For allyl alpha-ionone [FL-no: 07.061], two *in vitro* studies were submitted, a bacterial reverse mutation assay and a micronucleus assay in human peripheral blood lymphocytes. The bacterial mutation assay has been performed only in TA102 strain of *S. typhimurium*, in the presence and absence of S9 metabolic activation, where no indication of mutation has been observed. A study was previously performed in the other four strains of *S. typhimurium* and there was no indication of mutation after treatment with allyl alpha-ionone. Allyl alpha-ionone did not induce chromosomal damage or aneuploidy when tested with human peripheral blood lymphocytes in the absence and presence of S9 metabolic activation. This study is of limited validity due to deviations from the OECD Guideline 487 (OECD, 2010). In fact, the treatment of cells for 3 hours (with 21 hours recovery) in the absence of S9-mix was not performed. Therefore an *in vitro* micronucleus assay with treatment for 3 hours (with 21 hours recovery) in the absence of S9-mix should be performed.

alpha-Damascone [FL-no: 07.134] did not induce any significant increase in bacterial mutation frequency when evaluated in four histidine-requiring strains (TA98, TA100, TA1535 and TA1537) of *S. typhimurium* and *E. coli* WP2uvrA in presence and absence of metabolic activation.

alpha-Damascone did induce statistically significant chromosomal damage or aneuploidy when tested in the *in vitro* micronucleus test with human peripheral lymphocytes in the absence and presence of S9 metabolic activation. However, the results with alpha-damascone were difficult to interpret due to the difficulty in assessing the cytotoxicity of the test substance to the peripheral blood human lymphocytes. The Panel concluded that the study result was equivocal.

The current data available for alpha-damascone [FL-no: 07.134] cannot be used to exclude a genotoxic concern and accordingly the Panel requests additional data for this substance in order to conclude on the genotoxicity of this substance and the four structurally related substances [FL-no: 07.130, 07.225, 07.226 and 07.231].

Overall, the Panel concluded that the concern for genotox potential is ruled out for eight of the substances [FL-no: 02.105, 07.007, 07.009, 07.011, 07.036, 07.088, 07.091 and 07.170]. These eight substances can accordingly be evaluated using the Procedure. For allyl alpha-ionone [FL-no: 07.061] and for alpha-damascone [FL-no: 07.134] and the four structurally related substances [FL-no: 07.130, 07.225, 07.226 and 07.231], the new submitted data could not rule out the Panel concern with respect to genotoxicity and additional data are requested.

SUMMARY OF SPECIFICATION FOR SUBSTANCES IN FGE.210REV1 (JECFA, 1998; JECFA, 2005)

Table 2: Summary of Specification for the Substances in the Flavouring Group Evaluation 210Rev1

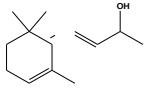
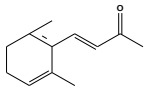
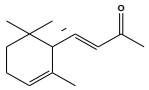
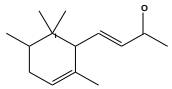
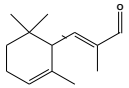
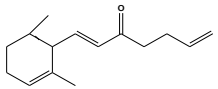
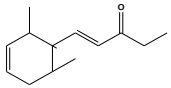
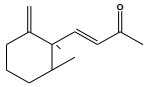
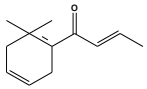
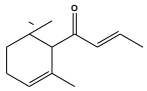
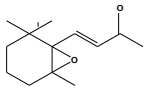
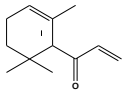
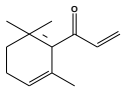
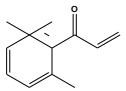
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
02.105 391	4-(2,6,6-Trimethyl-2-cyclohexenyl)but-3-en-2-ol		3624 25312-34-9	Liquid $C_{13}H_{22}O$ 194.32		127 (20 hPa) IR 99 %	1.488-1.492 0.917-0.924
07.007 388	alpha-Ionone		2594 141 127-41-3	Liquid $C_{13}H_{20}O$ 192.30	Insoluble 1 ml in 3 ml 70% alcohol	237 IR 85 %	1.497-1.502 0.927-0.933
07.009 398	Methyl-alpha-ionone		2711 143 7779-30-8	Liquid $C_{14}H_{22}O$ 206.33		238 IR 90 %	1.498-1.503 0.921-0.930
07.011 403	4-(2,5,6,6-Tetramethyl-2-cyclohexenyl)-3-buten-2-one		2597 145 79-69-6	Liquid $C_{14}H_{22}O$ 206.33	1 ml in 4 ml 70% alcohol	110-112 (4 hPa) IR 98 %	1.497-1.503 0.932-0.939
07.036 404	alpha-Isomethyl ionone		2714 169 127-51-5	Liquid $C_{14}H_{22}O$ 206.33		238 IR 85 %	1.498-1.503 0.925-0.934
07.061 401	Allyl alpha-ionone		2033 2040 79-78-7	Liquid $C_{16}H_{24}O$ 232.37	Insoluble 1 ml in 1 ml 90% alcohol	265 IR 88 %	1.502-1.507 0.926-0.935
07.088 400	Methyl-delta-ionone		2713 11852 7784-98-7	Liquid $C_{14}H_{22}O$ 206.33	Insoluble	232 IR 95 %	1.493-1.499 0.931-0.938

Table 2: Summary of Specification for the Substances in the Flavouring Group Evaluation 210Rev1

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)
07.091 390	gamma-Ionone		3175 79-76-5	Liquid $C_{13}H_{20}O$ 192.30		125 (13 hPa) NMR MS 95 %	1.496-1.502 (25°) 0.932-0.935 (20°)
07.130 386	delta-Damascone		3622 57378-68-4	Liquid $C_{13}H_{20}O$ 192.30	1 ml in 10 ml 95% alcohol	82 (3 hPa) IR 96.5 %	1.485-1.502 0.920 -0.940
07.134 385	alpha-Damascone		3659 11053 43052-87-5	Liquid $C_{13}H_{20}O$ 192.30		90-100 IR 98 %	1.492-1.499 0.928-0.938
07.170 1571	beta-Ionone epoxide		4144 11202 23267-57-4	Solid $C_{13}H_{20}O_2$ 208.30	Insoluble Soluble	 48 NMR MS 95 %	n.a. n.a.
07.225	cis-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one		3659 11053 23726-94-5	Liquid $C_{13}H_{20}O$ 192.3	Insoluble Soluble	 MS 92 %	1.492-1.499 0.928-0.938
07.226	tr-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one		 24720-09-0	Liquid $C_{13}H_{20}O$ 192.30	Freely soluble	54 (0.1 hPa) 95 %	1.493-1.499 0.937-0.943
07.231	alpha-Damascenone		 35044-63-4	Liquid $C_{13}H_{18}O$ 190.28	Practically insoluble or insoluble Freely soluble	51 (0.1 hPa) MS 95 %	1.502-1.508 1.015-1.021

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.

SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (JECFA, 1999; JECFA, 2006)

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

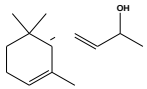
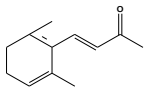
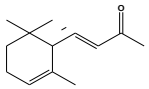
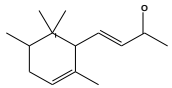
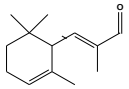
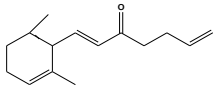
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (μg/capita/ day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]
02.105 391	4-(2,6,6-Trimethyl-2-cyclohexenyl)but-3-en-2-ol		0.61 0.06	Class I A3: Intake below threshold	4)	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
07.007 388	alpha-Ionone		270 150	Class I A3: Intake below threshold	4)	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
07.009 398	Methyl-alpha-ionone		86 7	Class I A3: Intake below threshold	4)	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
07.011 403	4-(2,5,6,6-Tetramethyl-2-cyclohexenyl)-3-buten-2-one		7.7 3	Class I A3: Intake below threshold	4)	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
07.036 404	alpha-Isomethyl ionone		4.7 1	Class I A3: Intake below threshold	4)	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
07.061 401	Allyl alpha-ionone		30 25	Class I A3: Intake below threshold	4)	Evaluated in FGE.210Rev1, genotoxicity concern could not be ruled out. Additional genotoxicity data required.

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

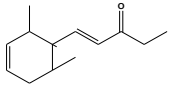
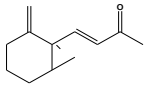
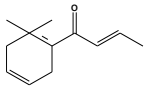
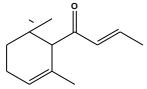
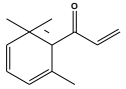
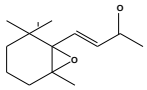
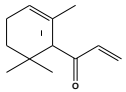
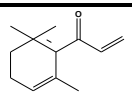
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (μg/capita/ day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]
07.088 400	Methyl-delta-ionone		0.37 1	Class I A3: Intake below threshold	4)	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
07.091 390	gamma-Ionone		0.012 15	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
07.130 386	delta-Damascone		0.049 0.6	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Evaluated in FGE.210Rev1, genotoxicity concern could not be ruled out. Additional genotoxicity data required.
07.134 385	alpha-Damascone		6.9 0.4	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Evaluated in FGE.210Rev1, genotoxicity concern could not be ruled out. Additional genotoxicity data required.
07.231	alpha-Damascenone		0.57	Class I No evaluation	Not evaluated by JECFA	Evaluated in FGE.210Rev1, genotoxicity concern could not be ruled out. Additional genotoxicity data required.
07.170 1571	beta-Ionone epoxide		0.073 0.1	Class III No evaluation	Not evaluated by JECFA	Evaluated in FGE.210Rev1, genotoxicity concern could be ruled out. Can be evaluated using the Procedure.
07.225	cis-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one		24	No evaluation	Not evaluated by JECFA	Evaluated in FGE.210Rev1, genotoxicity concern could not be ruled out.Additional genotoxicity data required.

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

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)]
07.226	tr-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one		0.011	No evaluation	Not evaluated by JECFA	Evaluated in FGE.210Rev1, genotoxicity concern could not be ruled out. Additional genotoxicity data required.

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.

QSAR PREDICTIONS ON MUTAGENICITY IN FIVE MODELS FOR 10 KETONES FROM SUBGROUP 2.4

Table 4: QSAR Predictions on Mutagenicity in Five Models for 12 Ketones from Subgroup 2.4

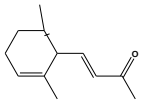
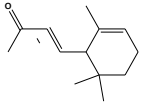
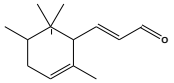
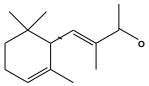
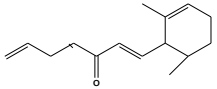
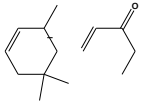
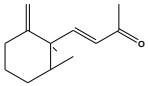
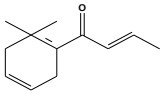
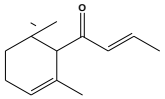
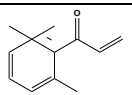
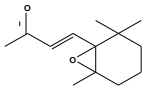
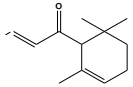
FL-no JECFA-no	EU Register name	Structural formula	ISS Local Model Ames Test TA100	MultiCASE Ames test	MultiCASE Mouse lymphoma test	MultiCASE Chromosomal aberration test in CHO	MultiCASE Chromosomal aberration test in CHL
07.007 388	alpha-Ionone		NEG	NEG	NEG	NEG	EQU
07.009 398	Methyl-alpha-ionone		NEG	NEG	OD	NEG	EQU
07.011 403	4-(2,5,6,6-Tetramethyl-2-cyclohexenyl)-3-buten-2-one		NEG	NEG	OD	NEG	EQU
07.036 404	alpha-Isomethyl ionone		NEG	NEG	NEG	NEG	NEG
07.061 401	Allyl alpha-ionone		NEG	NEG	NEG	NEG	EQU
07.088 400	Methyl-delta-ionone		NEG	NEG	OD	OD	EQU
07.091 390	gamma-Ionone		NEG	NEG	NEG	NEG	EQU
07.130 386	delta-Damascone		NEG	NEG	NEG	NEG	EQU

Table 4: QSAR Predictions on Mutagenicity in Five Models for 12 Ketones from Subgroup 2.4

FL-no JECFA-no	EU Register name	Structural formula	ISS Local Model Ames Test TA100	MultiCASE Ames test	MultiCASE Mouse lymphoma test	MultiCASE Chromosomal aberration test in CHO	MultiCASE Chromosomal aberration test in CHL
07.134 385	alpha-Damascone		NEG	NEG	OD	NEG	OD
07.231	alpha-Damascenone		NEG	NEG	OD	OD	OD
07.170	beta-Ionone epoxide		NYA	NEG	OD	OD	OD
07.226	tr-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one		NYA	NEG	NEG	NEG	OD

Column 3: Structure group 2.4: α,β-unsaturated ketones.
Column 4: Local model on aldehydes and ketones, Ames TA100. (NEG: Negative; POS: Positive; OD*: out of domain).
Column 5: MultiCase Ames test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
Column 6: MultiCase Mouse Lymphona test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
Column 7: MultiCase Chromosomal aberration in CHO (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
Column 8: MultiCase Chromosomal aberration in CHL (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
* OD, out of applicability domain: not matching the range of conditions where a reliable prediction can be obtained in this model. These conditions may be physicochemical, structural, biological etc.

GENOTOXICITY DATA (*IN VITRO*) CONSIDERED BY THE PANEL IN FGE.210

Table 5: Genotoxicity Data (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [c]
alpha-Ionone [07.007]	Chromosomal aberration	Chinese hamster B241 cell line	25 nmol/L	Positive[a]	(Kasamaki et al., 1982)	Limited validity (limited documentation; results for only one test concentration reported; long incubation period of 24 hours; unusual cell line)
	Reverse mutation	*S. typhimurium* TA98, TA100	0.01 - 50 µg/plate	Negative[a]	(Kasamaki et al., 1982)	Limited validity (insufficiently reported. Only two strains).
	Rec assay	*B. subtilis* H17 & M45	19 mg/disc	Negative[b]	(Oda et al., 1978)	Insufficient validity. This bacterial DNA-repair test system is of low predictive value for genotoxicity
Methyl-alpha-ionone [07.009]	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	5 concentrations up to cytotoxicity or max 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Limited validity (no TA102 or *E. Coli*)
Methyl-delta-ionone [07.088]	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	5 concentrations up to cytotoxicity or max 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Limited validity (no TA102 or *E. Coli*)

a: With and without metabolic activation
b: Activation status unknown
c: Validity of genotoxicity studies:
Valid
Limited validity (e.g. if certain aspects are not in accordance with OECD Guidelines or current standards and / or limited documentation)
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system)
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

GENOTOXICITY DATA (*IN VIVO*) CONSIDERED BY THE PANEL IN FGE.210

Table 6: Genotoxicity Data (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Reported Result	Reference	Comments a
Methyl alpha-ionone [07.009]	Micronucleus formation	NMRI mice, male and female, bone marrow	I.P.	825 - 2063 mg/kg bw	Negative	(Wild et al., 1983)	Limited validity (only analysis at one time point; no PCE/NCE ratio reported)
	Sex-linked recssive lethals	*Drosophila melanogaster*	Feed	20 mM	Negative		Limited validity (limited reporting, test system considered of limited relevance)

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

GENOTOXICITY DATA (*IN VITRO*) CONSIDERED BY THE PANEL IN FGE.210REV1

Table 7: Additional Genotoxicity Data (*in vitro*)

Chemical Name FL-no	Test System in vitro	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
alpha-Ionone [07.007]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	0.3 - 5000 µg/plate [1,3] 0.3 - 5000 µg/plate [2,3]	Negative Negative	(Bowen, 2011)	Toxicity was observed at 1000 and/or 5000 µg/plate across all strains in the absence and presence of S9-mix; no clear evidence of toxicity in TA100 in the presence of S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA98 TA100	 156.3 - 5000 µg/plate [2,4] 156.3 - 5000 µg/plate [1,3] or [2,4]	 Negative Negative		Evidence of toxicity was observed at the highest three or four concentrations across all strains in the absence and presence of S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA98 TA1535, TA1537 and TA102	 78.1 – 2500 µg/plate [1,3] 78.1 – 2500 µg/plate [1,3] or [2,4]	 Negative Negative		Evidence of toxicity was observed at the highest three or four concentrations across all strains in the absence or presence of S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA1535 TA102 TA1537	39.1 - 2500 µg/plate [1,3] or [2,4] 19.5 - 1250 µg/plate [1,3] or [2,4] 19.5 - 1250 µg/plate [2,4]	Negative		Evidence of toxicity was observed at the highest three or four concentrations across all strains in the absence or presence of S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.

Table 7: Additional Genotoxicity Data (*in vitro*)

Chemical Name FL-no	Test System in vitro	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
	Micronucleus Induction	Human peripheral blood lymphocytes	160 - 180 µg/ml [2,8] 40 - 65 µg/ml [1,9]	Negative Negative	(Lloyd, 2013b)	The MNBN cell frequencies in all treated cultures fell within the normal range. The study does not comply with OECD Guideline 487 therefore it has limited validity
Allyl alpha-ionone [07.061]	Reverse Mutation	*S. typhimurium* TA102	1.6 - 5000 µg/plate [1,3] or [2,3]	Negative	(Ballantyne, 2011)	No evidence of toxicity was observed at any concentration. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
			51.2 - 5000 µg/plate [1,3] or [2,4]	Negative		No evidence of toxicity was observed at any concentration. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
	Reverse Mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	Five concentrations up to cytotoxicity or max 3600 µg/plate [1,3]	Negative	(Wild et al., 1983)	Limited validity (no TA102 or *E. Coli*).
	Micronucleus Induction	Human peripheral blood lymphocytes	110 - 160 µg/ml [2,8] 25 - 38 µg/ml [1,9]	Negative Negative	(Lloyd, 2013a)	The MNBN cell frequencies in all treated cultures fell within the normal range. The study does not comply with OECD Guideline 487, therefore it has limited validity.
delta-Damascone [07.130]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	4.9 - 5000 µg/plate [4,5]	Negative	(Shinya, 2006)	Evidence of toxicity was observed at the top three or four concentrations tested. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA98, TA100, TA1535 and TA1537	2.4 - 78.1 µg plate [1,4] 9.8 - 313 µg/plate [2,4]	Negative		Evidence of toxicity was observed at the top concentration in all strains in the absence of S9-mix and at 156 µg/plate or above in the presence of S9-mix. The study complies with current recommendations for upper concentration limit inclusion. The study

Table 7: Additional Genotoxicity Data (*in vitro*)

Chemical Name FL-no	Test System in vitro	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
						included 3 replicate plates per concentration, and was GLP compliant.
		E. coli WP2 uvrA	4.9 – 5000 µg/plate [5,4]	Negative		Evidence of toxicity was observed at the top three or four concentrations tested. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		E. coli WP2 uvrA	2.4 - 78.1 µg/plate [1,4] 9.8 – 313 µg/plate [2,4]	Negative		Evidence of toxicity was observed at the highest concentration in the presence of S9-mix. The study complies with current recommendations for upper concentration limit inclusion. The study included 3 replicate plates per concentration, and was GLP compliant.
alpha-Damascone [07.134]	Reverse mutation	*S. typhimurium* TA98 and TA100	10 – 5000 µg/plate [5,3]	Negative	(Haddouk, 2001)	In TA98, slight to marked toxicity was observed at concentrations ≥ 100 µg/plate or 500 µg/plate with and without S9-mix, respectively. In TA100, toxicity was observed at concentrations ≥ 500 µg/plate with and without S9-mix.
		S. typhimurium TA1537, TA98	7.8 – 125 µg/plate [5,3] 15.6 – 250 µg/plate [2,4]	Negative Negative		Slight toxicity was observed in all strains. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA100 and TA1535	31.2 – 500 µg/plate [5,3] 31.2 – 500 µg/plate [2,4]	Negative Negative		Slight toxicity was observed in all strains. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA1535, TA1537, TA98 and TA100	15.6 – 250 µg/plate [1,3]	Negative		Slight toxicity was observed in all strains. No statistically significant increase in revertant numbers was seen at any concentration
		E. coli WP2uvrA	10 - 5000µg/plate [5,3]	Negative		Slight toxicity was observed at 2500 µg/plate and above without S9-mix.

Table 7: Additional Genotoxicity Data (*in vitro*)

Chemical Name FL-no	Test System in vitro	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
		E. coli WP2 uvrA	312.5 – 5000 µg/plate [5,3] 312.5 – 5000 µg/plate [1,3] or [2,4]	Negative Negative		Slight toxicity was observed only at the highest concentration tested without S9-mix. No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
	Micronucleus Induction	Human peripheral blood lymphocytes	9 - 22 µg/ml [1,8] 12 - 22 µg/ml [2,8] 5 - 10 µg/ml [1,9]	Negative Weakly positive Negative	(Lloyd, 2012)	Weakly positive result was obtained only in the 3+21 hours treatment in the presence of S9-mix. Study design complies with OECD Guideline 487.
			7.5 - 14 µg/ml [2,8,10] 14 - 20 µg/ml [2,8,9] 7.5 - 14 µg/ml [2,8,10]	Positive	(Whitwell, 2012)	Follow-up study to explore different methods of mixing and sample preparation to overcome the challenges in inconsistent cytotoxicity that results in difficulties in choosing concentrations for scoring of micronucleated binucleate cells. Experiment conducted only for 3+21 hours in the presence of S9-mix.
			10 - 18 µg/ml [2,8] 7 - 14 µg/ml [1,9]	Positive at high toxic concentrations only	(Lloyd, 2013c)	Positive results were obtained only at high toxic concentrations in both test conditions. Study is robust and complies with GLP.
beta-Ionone epoxide [07.170]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, and TA1537	5 - 500 µg/plate [5,3]	Negative	(Jones and Wilson, 1988)	No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA97a, TA98, TA100, TA1535	501, 1582 and 5000 µg/plate [1,3] 158, 501 and 1582 µg/plate [2,3]	Negative Negative	(Kringstad, 2005)	Evidence of toxicity was observed at the highest concentration in strain TA97a in the absence of S9-mix and in TA100 in the absence and presence of S9-mix. The study therefore complies with current recommendations for upper concentration limit inclusion. The study included 3 replicate plates per concentration, and was GLP compliant.

Table 7: Additional Genotoxicity Data (*in vitro*)

Chemical Name FL-no	Test System in vitro	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
		E. coli WP2uvrA	501, 1582 and 5000 µg/plate [1,3] 158, 501 and 1582 µg/plate [2,3]	Negative Negative		
	tk Mutation Induction	Mouse Lymphoma L5178Y T/K +/- 3.7.2c cells	200 - 900 µg/ml [5,6] 4.1 - 520 µg/ml [1,7]	Negative Negative	(Flanders, 2006)	A preliminary range-finder assay was conducted to establish maximum concentrations. Top concentrations in each arm of the study induced 77, 85, and 80% reductions in relative total growth. The study therefore complies with current recommendations.

[1] Without S9 metabolic activation.
[2] With S9 metabolic activation.
[3] Plate incorporation method.
[4] Pre-incubation with S9 method.
[5] With and without S9 metabolic activation.
[6] 4-hour treatment.
[7] 24-hour treatment.
[8] 3-hour treatment with 21-hour recovery.
[9] 24-hour treatment with 0-hour recovery.
[10] Standard treatment in larger-than-typical vessel

GENOTOXICITY DATA (*IN VIVO*) CONSIDERED BY THE PANEL IN FGE.210REV1

Table 8: Additional Genotoxicity Data (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Reported Result	Reference	Comments a
alpha-Ionone [07.007]	Micronucleus formation	Male and female mice	Intraperitoneal	300, 600 and 1200 mg/kg bw/day	Negative	(Krsmanovic and Huston, 2006)	Study complies with OECD Guideline 474. Evidence of bone marrow toxicity as evidenced by reductions in polychromatic erythrocytes observed at 24 hours after dosing and in a satellite group at the top dose 48 hours after dosing.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CA	Chromosomal Aberrations
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CHL	Chinese Hamster Lung (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
ID	Identity
IOFI	International Organization of the Flavor Industry
IR	Infrared spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MNBN	Micronucleated binucleate cells
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
MTD	Maximum Tolerated Dose
NCE	Normochromatic Erythrocytes
No	Number
NOAEL	No Observed Adverse Effect Level
PCE	Polychromatic Erythrocytes
OECD	Organisation for Economic Co-operation and Development
QSAR	Quantitative Structure-Activity Relationship
WHO	World Health Organisation