European Food Safety Authority

The EFSA Journal (2009) ON-1030, 1-18

SCIENTIFIC OPINION

Flavouring Group Evaluation 210: alpha,beta-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.4 of FGE.19[1]

Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2008-766)

Adopted on 29 January 2009

PANEL MEMBERS

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SUMMARY

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advise for 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 asked to evaluate flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation 210 (FGE.210) concerns 13 substances. The 13 substances correspond to subgroup 2.4 of FGE.19. Twelve of these substances are alpha,beta-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 is a precursor for such ketones [FL-no:

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Comission on Flavouring Group Evaluation 210: alpha,beta-Unsaturated aliphatic aldehydes and precursors from hemical subgroup 1.1.4 of FGE.19 with two or more conjugated double bonds and with or without additional non-conjugated double bonds. *The EFSA Journal* (2009) ON-1030, 1-18.

02.105]. One of the substances has a terminal double bond [FL-no: 07.061] and one is an epoxide [FL-no: 07.170].

No carcinogenicity studies are available for the 13 alpha,beta-unsaturated ketones in subgroup 2.4.

The genotoxicity concern with respect to the 13 alpha,beta-unsaturated alicyclic ketones and precursors in the present FGE.210 cannot be ruled out based on the genotoxicity data and the (quantitative) structure-activity relationship ((Q)SAR) predictions available.

Therefore, the Panel concluded that the substances cannot presently be evaluated through the Procedure. 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.

KEY WORDS: alpha,beta-Unsaturated ketones, flavouring substances, safety evaluation.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) 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 2008/478/EC (EC, 2008). 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, 1999). 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 community 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, 1996).

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 were considered by the Panel to be 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. The Panel decided that 11 subgroups (1.1.2, 1.1.3, 1.1.4, 2.4, 2.6, 2.7, 3.1, 3.3, 4.1, 4.2 and 4.4) (EFSA, 2008b) should be further examined to determine whether evaluation through the Procedure is feasible. Corresponding to these 11 subgroups 11 Flavouring Group Evaluations (FGEs) were established (FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220). If the Panel concludes for any substances in these 11

FGEs that they cannot be evaluated using the Procedure then it has to be decided if there is a safety concern for certain substances or if additional data are required in order to finalise the evaluation. If the Panel concludes that a genotoxic potential can be ruled out for the substances they will be merged with structurally related substances in other FGEs and evaluated using the Procedure.

TERMS OF REFERENCE

European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances prior to their authorisation and inclusion in a community list according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Riccardo Crebelli, Karl-Heinz Engel, Henrik Frandsen, Jørn Gry, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Catherine Leclercq, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin.

ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 210

1.1. Description

The present Flavouring Group Evaluation 210 (FGE.210) concerns 13 substances, which are listed in Table 1. The 13 substances correspond to subgroup 2.4 of FGE.19 (EFSA, 2008b). Twelve of these substances are alpha,beta-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 is a precursor for such ketones [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].

A summary of their current evaluation status by the JECFA is given in Table 2 (JECFA, 1999a; JECFA, 2007a).

The alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b). Accordingly, the available data on genotoxic or carcinogenic activity for the 12 alpha,beta-unsaturated ketones [FL-no: 07.007, 07.009, 07.011, 07.036, 07.061, 07.088, 07.091, 07.130, 07.134, 07.170, 07.226 and 07.231], corresponding to 13 substances in FGE.210, will be considered in this FGE.

The Panel has also taken into consideration the outcome of the predictions from five selected (Q)SAR models (Benigni & Netzeva, 2007a; Gry et al., 2007; Nikolov et al., 2007) on the 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]. The 12 ketones and their (Q)SAR predictions are shown in Table 3.

2. Toxicity

2.1. (Q)SAR Predictions

In Table 3 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 cells (CHO), -Chromosomal aberration test in Chinese hamster lung cells (CHL), 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 four substances were out of domain (See Table 3).

2.2. Carcinogenicity Studies

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

2.3. 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 Table 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.

2.4. 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. Conclusions

The Panel concluded that a genotoxic potential of the 13 alpha,beta-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 presently be evaluated through the Procedure. 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, 2008bb).

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 210 (JECFA, 1999A; JECFA, 2007A)

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 210 (JECFA, 1999a; JECFA, 2007a)

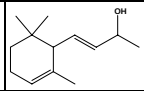
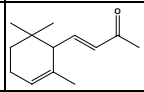
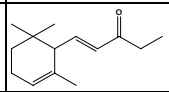
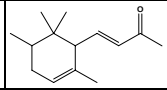
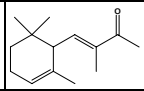
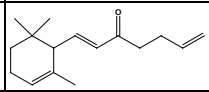
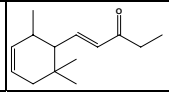
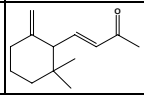
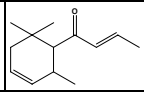
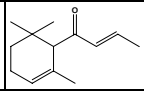
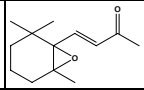
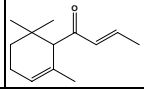
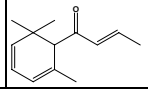
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
02.105 391	4-(2,6,6-Trimethyl-2-cyclohexenyl)but-3-en-2-ol		3624 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 1: Specification Summary of the Substances in the Flavouring Group Evaluation 210 (JECFA, 1999a; JECFA, 2007a)

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 98 %	1.492-1.499 0.928-0.938
07.170 1571	beta-Ionone epoxide		11202 23267-57-4	Solid $C_{13}H_{20}O_2$ 208.30	Insoluble Soluble	 48 NMR MS 95 %	n.a. n.a.
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	1 ml in 1 ml	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 1 ml in 1 ml	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.

n.a.: not applicable.

TABLE 2: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH) (JECFA, 1999A; JECFA, 2007A)

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (JECFA, 1999a; JECFA, 2007a)

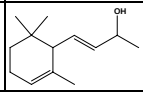
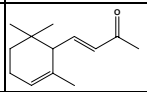
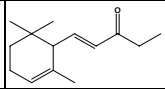
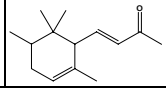
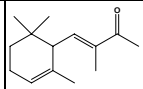
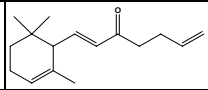
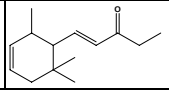
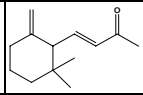
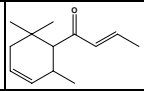
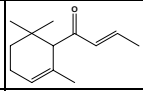
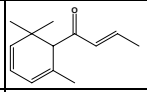
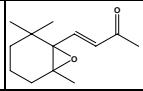
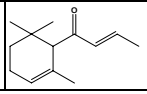
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (μg/*capita*/day) EU USA	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]
02.105 391	4-(2,6,6-Trimethyl-2-cyclohexenyl)but-3-en-2-ol		0.61 0.06	Class I A3: Intake below threshold	4)
07.007 388	alpha-Ionone		270 150	Class I A3: Intake below threshold	4)
07.009 398	Methyl-alpha-ionone		86 7	Class I A3: Intake below threshold	4)
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)
07.036 404	alpha-Isomethyl ionone		4.7 1	Class I A3: Intake below threshold	4)
07.061 401	Allyl alpha-ionone		30 25	Class I A3: Intake below threshold	4)
07.088 400	Methyl-delta-ionone		0.37 1	Class I A3: Intake below threshold	4)

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (JECFA, 1999a; JECFA, 2007a)

FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day) EU USA	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]
07.091 390	gamma-Ionone		0.012 15	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)
07.130 386	delta-Damascone		0.049 0.6	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)
07.134 385	alpha-Damascone		6.9 0.4	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)
07.231	alpha-Damascenone		0.57		Not evaluated by the JECFA.
07.170 1571	beta-Ionone epoxide		0.073 0.1	Class III A3: Intake below threshold	4)
07.226	tr-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one		0.011		Not evaluated by the JECFA.

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

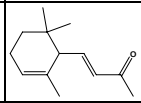
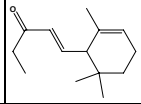
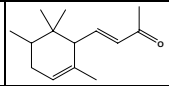
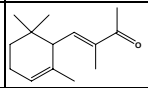
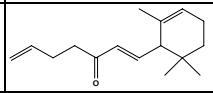
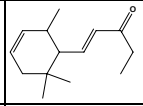
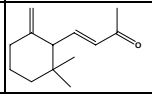
2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

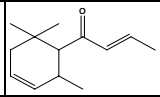
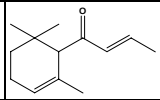
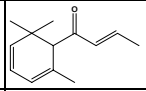
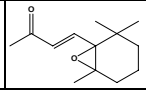
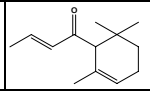
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

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

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

TABLE 3: (Q)SAR PREDICTIONS ON MUTAGENICITY IN FIVE MODELS FOR TWELVE KETONES FROM SUBGROUP 2.4

FL-no JECFA-no	Sub-group	EU Register name	Structural formula	FEMA no CoE no CAS no	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	2.4	alpha-Ionone		2594 141 127-41-3	NEG	NEG	NEG	NEG	EQU
07.009 398	2.4	Methyl-alpha-ionone		2711 143 7779-30-8	NEG	NEG	OD	NEG	EQU
07.011 403	2.4	4-(2,5,6,6-Tetramethyl-2-cyclohexenyl)-3-buten-2-one		2597 145 79-69-6	NEG	NEG	OD	NEG	EQU
07.036 404	2.4	alpha-Isomethyl ionone		2714 169 127-51-5	NEG	NEG	NEG	NEG	NEG
07.061 401	2.4	Allyl alpha-ionone		2033 2040 79-78-7	NEG	NEG	NEG	NEG	EQU
07.088 400	2.4	Methyl-delta-ionone		2713 11852 7784-98-7	NEG	NEG	OD	OD	EQU
07.091 390	2.4	gamma-Ionone		3175 - 79-76-5	NEG	NEG	NEG	NEG	EQU

FL-no JECFA-no	Sub-group	EU Register name	Structural formula	FEMA no CoE no CAS no	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
07.130 386	2.4	delta-Damascone		3622 - 57378-68-4	NEG	NEG	NEG	NEG	EQU
07.134 385	2.4	alpha-Damascone		3659 11053 43052-87-5	NEG	NEG	OD	NEG	OD
07.231	2.4	alpha-Damascenone		35044-63-4	NEG	NEG	OD	OD	OD
07.170	2.4	beta-Ionone epoxide		11202 23267-57-4	NYA	NEG	OD	OD	OD
07.226	2.4	tr-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one		24720-09-0	NYA	NEG	NEG	NEG	OD

Column 2: Structure group 2.4: alpha,beta-unsaturated alicyclic ketones.

Column 6: Local model on aldehydes and ketones, Ames TA100 (NEG: Negative; POS: Positive; OD: Out of domain; NYA: not yet assessed).

Column 7: MultiCASE Ames test (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).

Column 8: MultiCASE Mouse lymphoma test (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).

Column 9: MultiCASE Chromosomal aberration in CHO (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).

Column 10: 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.

TABLE 4: CARCINOGENICITY STUDIES

No carcinogenicity studies are available for the substances in subgroup 2.4 of FGE.19.

TABLE 5: GENOTOXICITY (*IN VITRO*)

Table 5: GENOTOXICITY (*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 hrs; 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).

TABLE 6: GENOTOXICITY (*IN VIVO*)

Table 6: GENOTOXICITY (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	825-2063 mg/kg bw	i.p.	Negative	(Wild et al., 1983)	Limited validity (only analysis at one time point; no PCE/NCE ratio reported).
	Sex-linked recessive lethals	*Drosophila melanogaster*	Feed	20 mM	Negative	(Wild et al., 1983)	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).

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ABBREVIATIONS

CAS	Chemical Abstract Service
CHL	Chinese hamster lung cell(s)
CHO	Chinese hamster ovary cell(s)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FL	Flavis
ID	Identity
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MS	Mass spectrometry
MSDI	Maximum Survey-derived Daily Intake
NMR	Nuclear magnetic resonance
No	number
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
(Q)SAR	(Quantitative) structure-activity relationship
SCF	Scientific Committee on Food
WHO	World Health Organisation