European Food Safety Authority

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SCIENTIFIC OPINION

Flavouring Group Evaluation 217: alpha,beta-Unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: Lactones[1]

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

(Question No EFSA-Q- 2008-762)

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 advice 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 217 (FGE.217) concerns 12 substances. The 12 substances correspond to subgroup 4.1 of FGE.19. Ten of the substances are alpha,beta-unsaturated lactones [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.046, 10.054, 10.060, 10.066 and 13.012], which by hydrolysis and oxidation give rise to alpha,beta-unsaturated ketones, and two substances [FL-no: 10.043 and 10.057] are precursors for the two alpha,beta-unsaturated ketones 2,7-dimethyl-4-oxo-oct-5,7-dienoic acid and 3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one, respectively.

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Material, Enzymes, Flavourings & Processing Aids on a request from the Commission on Flavouring Group Evaluation 217: alpha,beta-Unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: Lactones. *The EFSA Journal* (2009) ON-1068, 1-20.

6-Methylcoumarin [FL-no: 13.012] is not considered genotoxic and will therefore be allocated to FGE.80Rev1 for evaluation through the Procedure.

Based on the data available, a genotoxic potential of the remaining substances in the present FGE cannot be excluded. Therefore, the Panel concluded that they presently cannot be evaluated through the Procedure. Additional data on genotoxicity for representative substances of 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, lactones, 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 flavouring 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, 2008a). 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

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 217

1.1. Description

The present Flavouring Group Evaluation 217 (FGE.217) concerns 12 substances, which are presented in Table 1. These 12 substances correspond to subgroup 4.1 of FGE.19 (EFSA, 2008b). Ten of the substances are alpha,beta-unsaturated lactones [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.046, 10.054, 10.060, 10.066 and 13.012], which by hydrolysis and oxidation give rise to alpha,beta-unsaturated lactones, and two substances [FL-no: 10.043 and 10.057] are precursors for the two alpha,beta-unsaturated ketones 2,7-dimethyl-4-oxo-oct-5,7-dienoic acid and 3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one, respectively. Of these 12 substances 6-methylcoumarin [FL-no: 13.012] is the only substance in which the double bond in the alpha,beta-position is conjugated with an aromatic ring.

A summary of their current evaluation status by JECFA is given in Table 2 (JECFA, 1998a; JECFA, 2004b).

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 10 lactones in FGE.217 [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.046, 10.054, 10.060, 10.066 and 13.012] and the two alpha,beta-unsaturated ketones (2,7-dimethyl-4-oxo-oct-5,7-dienoic acid and 3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one), anticipated to be metabolism products from the two precursors in FGE.217 [FL-no: 10.043 and 10.057], 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 10 of the 12 lactones [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.046, 10.054, 10.060, 10.066 and 13.012] and the two alpha,beta-unsaturated ketones (2,7-dimethyl-4-oxo-oct-5,7-dienoic acid and 3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one – both non-Register substances) anticipated to be metabolism products formed from the two remaining lactones [FL-no: 10.043 and 10.057]. The 10 lactones and the two 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 or out of domain results for the Ames test system except for one positive prediction for 6-methylcoumarin [FL-no: 13.012].

For the predictions in the Mouse lymphoma test and the Chromosomal aberration test in CHO and CHL the results are inhomogeneous (in most cases either negative, out of domain or equivocal).

The only positive predictions are seen in the Mouse lymphoma test for the furan-2(5H)-one [FL-no: 10.066] and in the Chromosomal aberration test for hex-2-eno-1,4-lactone [FL-no: 10.046].

2.2. Carcinogenicity Studies

Groups of 25 male and 25 female weanling Osborne-Mendel rats were fed diets containing 0, 500, 1000, 3500, 5000, 7500 or 15000 mg/kg body weight (bw)/day 6-methylcoumarin [FL-no: 13.012] for two years, corresponding to 0, 25, 50, 175, 250, 375 or 750 mg 6-methylcoumarin/kg bw/day. Growth depression was observed in males at 375 mg 6-methylcoumarin/kg bw/day (moderate effect) and at 750 mg/kg bw/day (severe effect) paralleled by decreased food intake. In the liver, slight fatty metamorphosis and very slight bile duct proliferation was observed at the highest dose level. In addition, moderate testicular atrophy was seen in the high-dose males, presumably due to the severe growth depression. No other toxicological effects, including carcinogenicity, were seen. The Panel noted that in parallel studies the same research group was able to clearly demonstrate the liver carcinogenicity of safrole after dietary administration to rats (Hagan et al., 1967).

The Panel also noted that this study was performed before OECD test guidelines 451/453 (1981) were established and that it does not meet the criteria of these OECD test guidelines with respect to the number of animals. However, the Panel agreed with the conclusion of the authors that 6-methylcoumarin was not carcinogenic in rats under the study conditions.

Study validation and result are presented in Table 4.

2.3. Genotoxicity Studies

In subgroup 4.1 studies are available for one substance, 6-methylcoumarin [FL-no: 13.012], for which four *in vitro* and two *in vivo* study have been evaluated.

6-Methylcoumarin was found negative in two valid Ames tests (Brusick, 1982a; Haworth et al., 1983); equivocal results were obtained in a valid study with strain TA100 (Wild et al., 1983). It was found negative in a valid mouse lymphoma tk assay (Cifone, 1982a). Furthermore, it was found negative in the following three *in vivo* studies considered of limited validity: a *Drosophila melanogaster* sex-linked recessive lethal test (Wild et al., 1983), a mouse bone marrow micronucleus assay (Wild et al., 1983) and a mouse peripheral blood micronucleus 90-day assay reported by Witt et al. (2000).

Overall, the Panel concluded that the data available do not indicate a genotoxic potential for 6-methylcoumarin.

For the remaining 11 substances in FGE.217 no genotoxicity studies are available. Therefore, the genotoxic potential of these substances cannot be evaluated.

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

2.4. Conclusion on Genotoxicity and Carcinogenicity

The data available do not indicate a genotoxic or carcinogenic potential for 6-methylcoumarin. As the alpha,beta-unsaturated lactone 6-methylcoumarin is the only substance in this FGE with the alpha,beta-ketone grouping in conjugation with an aromatic ring, this substance would not be considered a representative for the remaining lactones in this group. The genotoxic potential of the other substances in this FGE cannot be evaluated.

3. Conclusions

6-Methylcoumarin [FL-no: 13.012] is not considered genotoxic and will therefore be allocated to FGE.80Rev1 for evaluation through the Procedure.

Based on the data available, a genotoxic potential of the remaining substances in the present FGE [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.043, 10.046, 10.054, 10.057, 10.060 and 10.066] cannot be excluded. Therefore, the Panel concluded that they presently cannot be evaluated through the Procedure. Additional data on genotoxicity for representative substances of 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 217 (JECFA, 1997B; JECFA, 2003B)

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 217 (JECFA, 1997b; JECFA, 2003b)

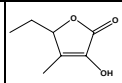
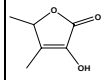
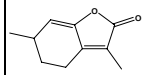
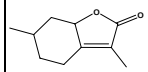
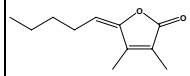
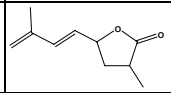
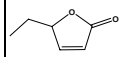
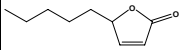
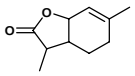
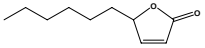
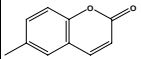
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
10.023 222	5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one		3153 2300 698-10-2	Liquid $C_7H_{10}O_3$ 142.15	Soluble	83-86 (1 hPa) IR 95 %	1.486-1.493 1.134-1.144
10.030 243	3-Hydroxy-4,5-dimethylfuran-2(5H)-one		3634 11834 28664-35-9	Liquid $C_6H_8O_3$ 128.13		81 (8 hPa) 25 IR 97.5 %	
10.034 1163	5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one		3755 80417-97-6	Liquid $C_{10}H_{12}O_2$ 164.20	Slightly soluble Soluble	264-266 (13hPa) IR NMR 95 %	1.542-1.548 1.090-1.096
10.036 1162	5,6,7,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4H)-one		3764 13341-72-5	Liquid $C_{10}H_{14}O_2$ 166.22	Slightly soluble Soluble	261-263 (8 hPa) IR NMR 98 %	1.497-1.503 1.058-1.063
10.042	3,4-Dimethyl-5-pentylidenefuran-2(5H)-one 6)		4050 11873 774-64-1	Liquid $C_{11}H_{16}O_2$ 180	Soluble 1 ml in 1 ml	303 MS 93 %	1.560-1.575 0.980-1.000
10.043	2,7-Dimethylocta-5(trans),7-dieno-1,4-lactone		 74183-60-1	Liquid $C_{10}H_{14}O_2$ 166.22	Practically insoluble or insoluble 1 ml in 1 ml	132 (8 hPa) NMR 95 %	1.453-1.459 0.977-0.983
10.046	Hex-2-eno-1,4-lactone		 2407-43-4	Liquid $C_6H_8O_2$ 112.13	 1 ml in 1 ml	93 (13 hPa) 95 %	1.431-1.437 1.067-1.073

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 217 (JECFA, 1997b; JECFA, 2003b)

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)
10.054	Non-2-eno-1,4-lactone		4188 21963-26-8	Liquid $C_9H_{14}O_2$ 154.21	Practically insoluble or insoluble 1 ml in 1 ml	196 95 %	1.457-1.463 0.981-0.987
10.057	3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3H)-one		4140 57743-63-2	Liquid $C_{10}H_{14}O_2$ 166.22	Practically insoluble or insoluble 1 ml in 1 ml	231 13 95 %	1.494-1.500 1.053-1.059
10.060	2-Decen-1,4-lactone		 2518-53-8	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble 1 ml in 1 ml	145 (13 hPa) MS 95 %	1.457-1.463 0.976-0.981
10.066	Furan-2(5H)-one		4138	Liquid $C_4H_4O_2$ 84.07	Soluble 1 ml in 1 ml	214 95 %	1.457-1.463 1.182-1.188
13.012 1172	6-Methylcoumarin		2699 579 92-48-8	Solid $C_{10}H_8O_2$ 160.17	Insoluble Soluble	 73-79 IR 99 %	n.a. n.a.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95% ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

TABLE 2: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH) (JECFA, 1998A; JECFA, 2004B)

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

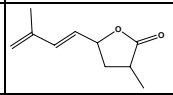
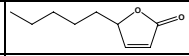
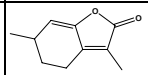
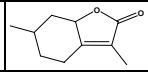
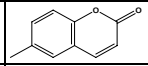
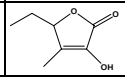
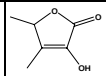
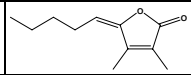
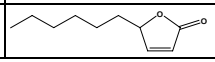
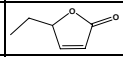
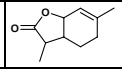
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (μg/*capita*/day) EU USA	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]
10.043	2,7-Dimethylocta-5(trans),7-dieno-1,4-lactone		0.0012	Class I	Not evaluated by the JECFA.
10.054	Non-2-eno-1,4-lactone		0.012	Class I	Not evaluated by the JECFA.
10.066	Furan-2(5H)-one		0.61	Class I	Not evaluated by the JECFA.
10.034 1163	5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one		1.5 9	Class III A3: Intake below threshold	4)
10.036 1162	5,6,7,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4H)-one		3.5 9	Class III A3: Intake below threshold	4)
13.012 1172	6-Methylcoumarin		250 96	Class III B3: Intake above threshold	Data must be available 5.
10.023 222	5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one		11 6.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)
10.030 243	3-Hydroxy-4,5-dimethylfuran-2(5H)-one		1.8 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)
10.042	3,4-Dimethyl-5-pentylidenefuran-2(5H)-one		0.12	Class III	Not evaluated by the JECFA.
10.060	2-Decen-1,4-lactone		0.037	Class III	Not evaluated by the JECFA.

Table 2: 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) EU USA	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]
10.046	Hex-2-eno-1,4-lactone		0.0024		Not evaluated by the JECFA.
10.057	3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3H)-one		0.012		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.*

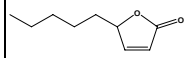
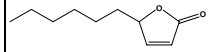
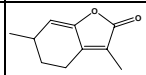
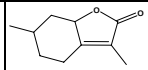
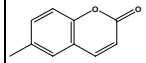
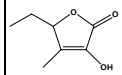
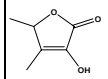
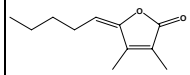
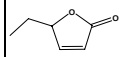
2) *Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.*

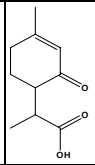
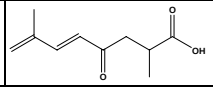
3) *Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.*

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: QSAR PREDICTIONS ON MUTAGENICITY IN FIVE MODELS FOR 16 LACTONES FROM SUBGROUP 4.1

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
10.054	4.1	Non-2-eno-1,4-lactone		4188 - 21963-26-8	OD*	NEG	OD*	EQU	OD*
10.060	4.1	2-Decen-1,4-lactone		- - 2518-53-8	OD*	NEG	OD*	EQU	OD*
10.066	4.1	Furan-2(5H)-one		4138 -	OD*	NEG	POS	EQU	EQU
10.034 1163	4.1	5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one		3755 - 80417-97-6	OD*	NEG	OD*	OD*	OD*
10.036 1162	4.1	5,6,7,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4H)-one		3764 - 13341-72-5	OD*	NEG	OD*	OD*	OD*
13.012 1172	4.1	6-Methylcoumarin		2699 579 92-48-8	OD*	POS	OD*	OD*	OD*
10.023 222	4.1	5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one		3153 2300 698-10-2	OD*	NEG	NEG	NEG	NEG
10.030 243	4.1	3-Hydroxy-4,5-dimethylfuran-2(5H)-one		3634 11834 28664-35-9	OD*	NEG	NEG	NEG	NEG
10.042	4.1	3,4-Dimethyl-5-pentylidenefuran-2(5H)-one		4050 11873 774-64-1	OD*	OD*	OD*	OD*	OD*
10.046	4.1	Hex-2-eno-1,4-lactone		- - 2407-43-4	OD*	NEG	OD*	POS	OD*

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
Not in Register	2.6	3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one		- - -	OD*	NEG	OD*	NEG	EQU
Not in Register	1.2.4	2,7-dimethyl-4-oxo-oct-5,7-dienoic acid		- - -	NYA	NYA	NYA	NYA	NYA

Column 2: Structure group 1.1.3: Aliphatic acyclic alpha,beta-unsaturated 3-alkylated aldehydes.

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; NYA: not yet assessed).*

Column 8: MultiCase Mouse lymphoma test (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal; NYA: not yet assessed).*

Column 9: MultiCase Chromosomal aberration in CHO (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal; NYA: not yet assessed).*

Column 10: MultiCase Chromosomal aberration in CHL (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal; NYA: not yet assessed).*

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.

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TABLE 4: CARCINOGENICITY STUDIES

Table 4: Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	Results	Reference	Comments
6-Methylcoumarin [13.012]	Rat; Male, Female 25/sex/group	Diet	0, 25, 50, 175, 250, 375 or 750 mg/kg bw/day	2 years	Males and females: No increases in tumour incidences	(Hagan et al., 1967)	The study is not in accordance with OECD Guidelines or current standards. Under the condition of the study the negative result is considered valid. The NOAEL was 250 mg/kg bw/day based on growth depression and slight liver changes particularly in males at the higher dose levels. The study is reported together with the results of studies of many more flavouring substances with and without related structures. Therefore no detailed description of the findings is given.

TABLE 5: GENOTOXICITY (*IN VITRO*)

Table 5: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments [d]
6-methylcoumarin [13.012]	Reverse mutation	S. *typhimurium* TA100	5 concentrations up to cytotoxicity, or max (≤3600 µg/plate)	Marginally positive[c]	(Wild et al., 1983)	Valid, however the results are considered equivocal (+ S9: dose-response showed positive trend, but was never above twice control frequency; - S9: negative).
	Reverse mutation	*S. typhimurium* TA98, TA1535, TA1537, and TA1538	5 concentrations up to cytotoxicity, or max. 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Valid.
	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, and TA1537	33–3333 µg/plate	Negative[a,b]	(Haworth et al., 1983)	Valid.
	Reverse mutation	S. *typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	1–5000 µg/plate	Negative[a]	(Brusick, 1982a)	Valid. Unpublished GLP study carried out according to current OECD guideline; result is considered as valid.
	Forward mutation	Mouse lymphoma L5178Y *Tk* +/-cells	6.25–100 µg/ml	Negative[c]	(Cifone, 1982a)	Valid. Unpublished GLP study carried out according to current OECD guideline; result is considered as valid.
	Forward mutation	Mouse lymphoma L5178Y *Tk* +/-cells	15.6–250 µg/ml	Negative	(Cifone, 1982a)	Valid. Unpublished GLP study carried out According to current OECD guideline; result is considered as valid.

a: With and without metabolic activation.

b: Pre-incubation method.

c: With metabolic activation.

d: 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	Result	Reference	Comments [a]
6-methylcoumarin [13.012]	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	Feed	10 mmol/l (1602 µg/ml)	Negative	(Wild et al., 1983)	Limited validity (limited reporting, study system considered of limited relevance).
	Micronucleus formation	Mouse peripheral blood cells	Oral (Gavage)	200 and 400 mg/kg for 90 days	Equivocal (M) Negative (F)	(Witt et al., 2000)	Limited validity (not a standard protocol; exposure for 90 days; no information on cytotoxicity; no positive controls).
	Micronucleus formation	Mouse bone-marrow cells	i.p.	160, 240, and 320 mg/kg	Negative	(Wild et al., 1983)	Limited validity (only analysis at one time point; no PCE/NCE ratio reported).

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
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
FLAVIS (FL)	Flavour Information System database
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