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Flavouring Group Evaluation 217 Revision 3 (FGE.217Rev3): consideration of genotoxic potential for α,β-unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: lactones

EFSA Panel on Food Additives and Flavourings (FAF),
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Abstract

The Panel on Food Additives and Flavourings of the European Food Safety Authority was requested to evaluate the genotoxic potential of four flavouring substances [FL-no: 10.023, 10.030, 10.057 and 13.012] from subgroup 4.1 of FGE.19. For three of these substances [FL-no: 10.023, 10.030 and 13.012], the concern for genotoxicity has been ruled out in previous revisions of Flavouring Group Evaluation 217 (FGE.217). However, in FGE.217Rev2, a concern for genotoxicity could not be ruled out for 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one [FL-no: 10.057]. After publication of FGE.217Rev2, industry provided additional genotoxicity studies for [FL-no: 10.057], which are evaluated in the present opinion FGE.217Rev3. The flavouring substance [FL-no: 10.057] did not induce gene mutations or numerical or structural chromosomal aberrations *in vitro*. Based on these data, the Panel concluded that the concern for genotoxicity is ruled out for [FL-no: 10.057]. Consequently, it can be evaluated through the Procedure.

Keywords: FGE.217, α,β-unsaturated ketones, lactones, flavouring substances, subgroup 4.1, FGE.19

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Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The use of flavourings is regulated under Regulation (EC) No 1334/2008[1] 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[2]. The list includes a number of flavouring substances for which the safety evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

In December 2018, EFSA FAF Panel adopted the opinion on FGE.217 Revision 2 that includes the flavouring substance 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2*(3H)*-one [FL-no: 10.057] represented by 3,4-dimethyl-5-pentylidenefuran-2(5*H*)-one [FL-no: 10.042] (FGE.217Rev2). For the representative substance 3,4-dimethyl-5-pentylidenefuran-2(5*H*)-one [FL-no: 10.042], the FAF Panel concluded that the potential clastogenicity at the site of contact should be further investigated through an *in vivo* comet assay in duodenum. [FL-no: 10.042] is also aneugenic *in vitro* and for such substances, there was no agreed follow-up strategy to finalise their safety assessment. Therefore, the Panel concluded that the substance [FL-no: 10.042] and the other eight represented substances [FL-no: 10.034, 10.036, 10.043, 10.046, 10.054, 10.057, 10.060 and 10.170] cannot be evaluated through the Procedure.

Following this evaluation there was an indication that the applicants were no longer interested to support the evaluation of the representative substance [FL-no: 10.042] and the other 8 substances, including [FL-no: 10.057]. Therefore, these substances were flagged for deletion from the Union List. However, early in 2021 the company Takasago indicated that they would support the evaluation of the substance [FL-no: 10.057]. Since the representative substance is no longer supported, in September 2021, they have provided the relevant data for the substance [FL-no: 10.057].

Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to evaluate the new information submitted and, depending on the outcome, proceed to the full evaluation of the substance 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one [FL-no: 10.057] in accordance with Commission Regulation (EC) No 1565/2000.

In case the genotoxic potential cannot be ruled out, EFSA is asked to estimate the exposure.

2. Data and methodologies

2.1. History of the evaluation of FGE.19 substances

Flavouring Group Evaluation 19 (FGE.19) contains 360 flavouring substances from the EU Register[4] 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. The Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC 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.

[1] 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.

[2] 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.

[3] 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 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8–16.

[4] Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996. OJ L 84, 27.3.1999, p. 1–137.

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 that were available at that time (DEREKfW, TOPKAT, DTU-NFI-MultiCASE Models and ISS-Local Models (Gry et al., 2007)).

The AFC 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 α,β-unsaturated carbonyls. Therefore, the AFC 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 AFC Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni and Netzeva, 2007a,b) 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 AFC 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 AFC 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 were 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). In selecting the representative substances, expert judgement was applied. In each subgroup, the representative substances were selected taking into account chain length, chain branching, lipophilicity and additional functional groups. 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 was requested to submit additional genotoxicity data according to the list of representative substances and test strategy for each subgroup.

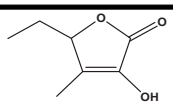
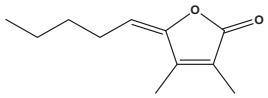
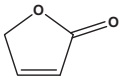
The Flavouring Industry has now submitted additional data and the present FGE concerns the evaluation of these data requested on genotoxicity.

2.2. History of the evaluation of the substances in subgroup 4.1

In the first scientific opinion on Flavouring Group Evaluation 217 (FGE.217), the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) concluded that additional genotoxicity data were required for 11 of the 12 α,β-unsaturated lactones considered in the FGE (EFSA, 2009). For one substance, 6-methylcoumarin [FL-no: 13.012], the concern for genotoxicity could be ruled out and accordingly the substance was evaluated using the Procedure in FGE.80Rev1 (EFSA CEF Panel, 2009). As 6-methylcoumarin is the only substance in FGE.217 with the α,β-ketone grouping in conjugation with an aromatic ring, the genotoxicity data for this substance could not be used for reading across to any of the remaining α,β-unsaturated lactones in this subgroup.

In the EFSA opinion 'List of α,β-unsaturated aldehydes and ketones representative of FGE.19 substances for genotoxicity testing' (EFSA, 2008c), three representative flavouring substances were selected (Table 1) for the remaining 11 substances of FGE.19, subgroup 4.1, corresponding to FGE.217. 5-Ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one [FL-no: 10.023] is a representative for the structurally related substance 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one [FL-no: 10.030]. Furan-2(5*H*)-one [FL-no 10.066] is considered as a stand-alone substance and 3,4-dimethyl-5-pentylidenefuran-2(5*H*)-one [FL-no: 10.042] is representative of the remaining seven substances [FL-no: 10.034, 10.036, 10.043, 10.046, 10.054, 10.057 and 10.060].

Table 1: Representative substances selected by EFSA for FGE.19 Subgroup 4.1 (EFSA, 2008c)

FL-no JECFA-no	EU Register[4] name	Structural formula
10.023 222	5-Ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one	
10.042 2002	3,4-Dimethyl-5-pentylidenefuran-2(5*H*)-one	
10.066 2000	Furan-2(5*H*)-one	

FGE: Flavouring Group Evaluation; FL-no: Flavour Information System; JECFA: The Joint FAO/WHO Expert Committee on Food Additives.

The CEF Panel considered that a genotoxic potential of the remaining 11 substances in FGE.217 [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] could not be excluded. Therefore, the CEF Panel concluded that additional data on genotoxicity for the representative substances [FL-no: 10.023, 10.042, 10.066] of this subgroup should be provided according to the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19 (EFSA, 2008b).

A revision of FGE.217 (FGE.217Rev1) was prepared due to additional data submitted by industry for the three representative substances 5-ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one [FL-no: 10.023], dimethyl-5-pentylidenefuran-2(5*H*)-one [FL-no: 10.042] and furan-2(5*H*)-one [FL-no: 10.066]. Based on the new data, the CEF Panel concluded that the genotoxicity concern could be ruled out for [FL-no: 10.023] and the one structurally related substance [FL-no: 10.030] for which it is a representative. Since these two substances were evaluated by JECFA before the year 2000 (JECFA, 1999), according to Commission Regulation (EC) No 1565/2000[3], there was no need for EFSA to further evaluate these substances following the Procedure (see also Appendix B, Table B.1). For the representative substances [FL-no: 10.042, and 10.066], the concern for genotoxicity could not be ruled out and a combined micronucleus and comet assay was requested for these two substances, covering the remaining seven substances [FL-no: 10.034, 10.036, 10.043, 10.046, 10.054, 10.057 and 10.060] (EFSA CEF Panel, 2013).

In FGE.217Rev2 (EFSA FAF Panel, 2019), the substance 5-pentyl-3*H*-furan-2-one [FL-no: 10.170] was added to the group. This substance is a mixture of two structural isomers, 2/3rds is the named substance (5-pentyl-3*H*-furan-2-one) and 1/3rd is the structural isomer 5-pentyl-5*H*-furan-2-one. This latter isomer is identical to [FL-no: 10.054], represented by [FL-no: 10.042] for which genotoxicity data were provided. In FGE.217Rev2, the FAF Panel evaluated new genotoxicity studies for furan-2(5*H*)-one [FL-no: 10.066] and 3,4-dimethyl-5-pentylidenefuran-2(5*H*)-one [FL-no: 10.042].

Based on these new data, the Panel concluded that furan-2(5*H*)-one [FL-no: 10.066] is genotoxic *in vivo*, and therefore, it was not evaluated according to the Procedure. The substance [FL-no: 10.066] was removed from the Union List.[5]

In the same opinion, FGE.217Rev2 (EFSA FAF Panel, 2019), the Panel concluded that for 3,4-dimethyl-5-pentylidenefuran-2(5*H*)-one [FL-no: 10.042], additional data would be needed to investigate the potential clastogenicity at the site of contact and also the potential aneugenicity. Therefore, in FGE.217Rev2, the Panel concluded that [FL-no: 10.042] and the other eight represented substances [FL-no: 10.034, 10.036, 10.043, 10.046, 10.054, 10.057, 10.060 and 10.170] could not be evaluated through the Procedure.

Industry communicated that they have no further interest in the evaluation of [FL-no: 10.042] and of the represented substances except [FL-no: 10.057], for which new genotoxicity studies have been provided. These studies are evaluated in the present opinion FGE.217Rev3.

[5] Commission Regulation (EU) 2019/799 of 17 May 2019 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards the removal from the Union list of the flavouring substance furan-2(5H)-one. OJ L 132, 20.5.2019, p. 12–14.

Consequently, the EU Commission has removed eight substances [FL-no: 10.034, 10.036, 10.042, 10.043, 10.046, 10.054, 10.060 and 10.170] from the Union List.[6] These substances and [FL-no: 10.066] will not be considered any further in FGE.217Rev3.

For the sake of completeness, the information on identity of all substances is maintained in Appendix B (summary of safety evaluation by JECFA). Information on specifications is only maintained for the substances which are currently in the Union List (see Appendix A). For substances that are no longer in the Union List, FGE.217Rev2 should be consulted.

Table 2 gives information on adoption dates and links to the published scientific opinions.

Table 2: Adoption dates and links to the published versions of FGE.217

FGE	Adopted	Link	No. of substances
FGE.217	29 January 2009	https://www.efsa.europa.eu/en/efsajournal/pub/1068	12
FGE.217Rev1	4 July 2013	https://www.efsa.europa.eu/en/efsajournal/pub/3304	12
FGE.217Rev2	11 December 2018	https://www.efsa.europa.eu/en/efsajournal/pub/5568	13
FGE.217Rev3	22 March 2023	https://www.efsa.europa.eu/en/efsajournal/pub/7967	4

FGE: Flavouring Group Evaluation.

2.3. Presentation of the substances in flavouring group evaluation 217Rev3

The present opinion FGE.217Rev3 concerns four substances, which are presented in Appendix A, Table A.1. These four substances correspond to subgroup 4.1 of FGE.19 (EFSA, 2008b). Three substances are α,β-unsaturated lactones [FL-no: 10.023, 10.030 and 13.012], which by hydrolysis and oxidation give rise to α,β-unsaturated ketones, and one substance [FL-no: 10.057] is a precursor for the α,β-unsaturated ketone 3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one. 6-Methylcoumarin [FL-no: 13.012] is the only substance in which the double bond in the α,β-position is conjugated with an aromatic ring. Since the concern for genotoxicity of three of these substances was already ruled out in previous versions of this FGE (EFSA, 2009; EFSA CEF Panel, 2013), the current revision 3 will only consider the additional data submitted for the flavouring substance [FL-no: 10.057].

The substances [FL-no: 10.023, 10.030, 10.057 and 13.012] have already been evaluated by JECFA. A summary of their current evaluation status by JECFA is given in Appendix B, Table B.1 (JECFA, 1999, 2004, 2016a).

The Panel has also taken into consideration the outcome of the predictions from five selected (Q) SAR models (Benigni and Netzeva, 2007a; Gry et al., 2007; Nikolov et al., 2007) on the anticipated metabolite (3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one, non-Register[4] substance), resulting from hydrolysis of the lactone moiety of [FL-no: 10.057] and subsequent oxidation of the liberated hydroxy group (see Appendix C, Table C.1).

3. Assessment

3.1. Additional data evaluated by the Panel in FGE.217Rev3

Industry submitted new genotoxicity data on [FL-no: 10.057]: a bacterial reverse mutation assay (Documentation provided to EFSA No. 3) and an *in vitro* micronucleus assay (Documentation provided to EFSA No. 4). These studies are evaluated in the present opinion FGE.217Rev3.

3.1.1. Bacterial reverse mutation assay with [FL-no: 10.057]

A bacterial reverse mutation assay was conducted in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and in *Escherichia coli* WP2 uvrA to assess the mutagenicity of [FL-no: 10.057] (purity > 97.7%), both in the absence and in the presence of metabolic activation by liver S9 fraction (S9-mix) from rats induced by phenobarbital and 5,6-benzoflavone. A concentration range finding test (designed as a full experiment with maximum concentrations up to 5,000 μg/plate in duplicate, for all strains and

[6] Commission Regulation (EU) 2022/1466 of 5 September 2022 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards the removal of certain flavouring substances from the Union list. OJ L 231, 6.9.2022, p. 32–35.

test conditions, including monitoring of induction of revertants and including positive controls) and one main experiment were conducted, using the pre-incubation method (Documentation provided to EFSA No. 3). In the range finding study there was no biologically relevant increase in revertants.

In the absence of S9-mix, concentrations from 156 μg/plate to 5,000 μg/plate were applied in the main experiment. Bacterial growth inhibition was observed from 2,500 μg/plate in all *S*. Typhimurium strains and only at the highest concentration in *E. coli* WP2 uvrA. In the presence of S9-mix, concentrations from 39 μg/plate to 1,250 μg/plate were applied for all *S*. Typhimurium strains and up to 5,000 μg/plate for *E. coli* WP2 uvrA. Bacterial growth inhibition was observed at the highest concentration tested in all bacterial strains. No precipitate was observed and no increase in the mean number of revertant colonies was observed at any tested concentration in any tester strain in the absence or presence of metabolic activation.

The study design complies with OECD Test Guideline (TG) 471 (OECD, 1997, 2020) and with the GLP principles, except that the test was conducted in duplicate plates instead of triplicate. However, the variability between the duplicate cultures in the range finding experiment and in the main experiment was small. In addition, the range finding test was designed as a full experiment and contributes therefore fully to the interpretation of the study results. The positive controls performed as expected, confirming the sensitivity of the test system. Therefore, this study is considered reliable without restrictions and it is concluded that the study result is negative.

3.1.2. *In vitro* mammalian cell micronucleus test with [FL no: 10.057]

Human peripheral blood lymphocytes from healthy donors were treated with [FL-no: 10.057] (purity 97.3%). The *in vitro* micronucleus assay was carried out according to OECD TG 487 (OECD, 2016) and GLP principles. The cytokinesis block micronucleus assay protocol was applied. Positive controls were cyclophosphamide, mitomycin C and vinblastine. DMSO was used as negative (vehicle) control (Documentation provided to EFSA No. 4).

For the main experiment, concentrations were selected based on the results of a range-finding test. Lymphocytes were treated with [FL-no: 10.057] at concentrations ranging from 463 to 1700 μg/mL in the 4 h treatment both in the absence and in the presence of metabolic activation (S9-mix from rats treated with Aroclor 1254) and from 40 to 463 μg/mL in the 24 h treatment in the absence of S9-mix. Cytotoxicity evaluation was based on cytokinesis-block proliferation index (CBPI). In the treatment of 4 h + 20 h in the presence of S9-mix, the following concentrations were chosen for MN analysis: 700, 864 and 1241 μg/mL (cytotoxicity of 19%, 24% and 51%, respectively). In the treatment of 4 h + 20 h in the absence of S9-mix, the following concentrations were chosen for MN analysis: 463, 813 and 1239 μg/mL (cytotoxicity of 13%, 26% and 52%, respectively). In the treatment of 24 h in the absence of S9-mix, the following concentrations were chosen for MN analysis: 40, 94 and 234 μg/mL (cytotoxicity of 15%, 20% and 53%, respectively). The substance [FL-no: 10.057] did not increase the frequency of micronucleated cells compared to vehicle (DMSO) controls in any of the testing conditions. The positive controls performed as expected, confirming the sensitivity of the test system. The Panel considered the results of this study to be negative.

4. Discussion

Following the publication of FGE.217Rev2, industry has submitted additional data for [FL-no: 10.057]. These new experimental data show that the substance [FL-no: 10.057] did not induce gene mutations or numerical or structural chromosomal aberrations *in vitro*. Therefore, no *in vivo* follow-up testing is needed (according to EFSA Scientific Committee, 2011).

The Panel concluded that the concern for genotoxicity of the substance [FL-no: 10.057] related to the α,β-unsaturated carbonyl metabolite 3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one, that could result from its hydrolysis and subsequent oxidation (see section 2.3) is ruled out. The substance [FL-no: 10.057] can now be evaluated through the Procedure in FGE.80Rev2.

5. Conclusions

The Panel concluded that the concern for genotoxicity is ruled out for [FL-no: 10.057], which can now be evaluated through the Procedure in FGE.80Rev2.

6. Documentation as provided to EFSA

1) Benigni R and Netzeva T, 2007a. Report on a QSAR model for prediction of genotoxicity of alpha,beta-unsaturated aldehydes in *S. typhimurium* TA100 and its application for predictions on alpha,beta-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.
2) Benigni R and Netzeva T, 2007b. Report on a QSAR model for prediction of genotoxicity of alpha,beta-unsaturated ketones in *S. typhimurium* TA100 and its application for predictions on alpha,beta-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.
3) [redacted], 2011. Mutagenicity test of wine lactone using microorganisms. Study number 15312. April 2011. Unpublished final report submitted by Takasago International Corporation.
4) [redacted], 2021. *In vitro* micronucleus assay in cultured human peripheral blood lymphocytes. Testing facility study No. 01557002. July 2021. Unpublished final report submitted by Takasago International Corporation.
5) Gry J, Beltoft V, Benigni R, Binderup M-L, Carere A, Engel K-H, Gürtler R, Jensen GE, Hulzebos E, Larsen JC, Mennes W, Netzeva T, Niemelä J, Nikolov N, Nørby KK and Wedebye EB, 2007. Description and validation of QSAR genotoxicity models for use in evaluation of flavouring substances in Flavouring Group Evaluation 19 (FGE.19) on 360 alpha,beta-unsaturated aldehydes and ketones and precursors for these. Unpublished report submitted by FLAVIS Secretariat to EFSA.
6) Nikolov N, Jensen GE, Wedebye EB and Niemelä J, 2007. Report on QSAR predictions of 222 alpha,beta-unsaturated aldehydes and ketones from Flavouring Group Evaluation 19 (FGE.19) on 360 alpha,beta-unsaturated aldehydes and ketones and precursors for these. Unpublished report submitted by FLAVIS Secretariat to EFSA.

References

EFSA (European Food Safety Authority), 2008a. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food held in Parma on 27–29 November 2007.

EFSA (European Food Safety Authority), 2008b. Genotoxicity Test Strategy for Substances belonging to Subgroups of FGE.19. Statement of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). EFSA Journal 2008;6(12):854, 5 pp. https://doi.org/10.2903/j.efsa.2008.854

EFSA (European Food Safety Authority), 2008c. List of alpha, beta-unsaturated aldehydes and ketones representative of FGE.19 substances for genotoxicity testing. Statement of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). EFSA Journal 2008;6(12):910, 7 pp. https://doi.org/10.2903/j.efsa.2008.910

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on Flavouring Group Evaluation 217: alpha, beta-unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: Lactones. EFSA Journal 2009;7(4):1068, 20 pp. https://doi.org/10.2903/j.efsa.2009.1068

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2009. Scientific opinion on Flavouring Group Evaluation 80, Revision 1 (FGE.80Rev1): consideration of alicyclic, alicyclic-fused and aromatic-fused ring lactones evaluated by JECFA (61st meeting) structurally related to a aromatic lactone evaluated by EFSA in FGE.27 (2008). EFSA Journal 2009;7(9):1169, 32 pp. https://doi.org/10.2903/j.efsa.2009.1169

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific Opinion on Flavouring Group Evaluation 217, Revision 1 (FGE.217Rev1). Consideration of genotoxic potential for α,β-unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: Lactones. EFSA Journal 2013;11(7):3304, 28 pp. https://doi.org/10.2903/j.efsa.2013.3304

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), 2019. Scientific Opinion on Flavouring Group Evaluation 217 Revision 2 (FGE.217Rev2), consideration of genotoxic potential for α, β-unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: lactones. EFSA Journal 2019;17(1):5568, 45 pp. https://doi.org/10.2903/j.efsa.2019.5568

EFSA Scientific Committee, 2011. Scientific opinion on Genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Safety evaluation of certain food additives and contaminants. Forty-ninth Meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 40. IPCS, WHO, Geneva.

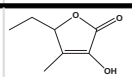
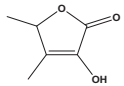
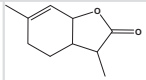
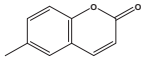
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Committee of Food Additives. Fifty-fifth Meeting. Geneva, 6–15 June 2000.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Committee of Food Additives 61st session. Rome, 10–19 June 2003.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2004. Safety evaluation of certain food additives and contaminants. Sixty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 52. IPCS, WHO, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008. Evaluation of certain food additives. Sixty-night report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series: 952,. WHO, Rome.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2011. Evaluation of certain food additives and contaminants. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series: 960. WHO, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2016a. Evaluation of certain food additives. Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series: 1000. WHO, Geneva.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2016b. Compendium of food additive specifications. Joint FAO/WHO Expert Committee on Food Additives eighty-second meeting.
OECD (Organisation for Economic Co-operation and Development), 1997. Test Guideline No. 471: Bacterial Reverse Mutation Test. OECD Guidelines for the Testing of Chemicals, Section 4.
OECD (Organisation for Economic Co-operation and Development), 2016. Test Guideline No. 487. *In Vitro* Mammalian Cell Micronucleus Test. OECD Guidelines for the Testing of Chemicals, Section 4.
OECD (Organisation for Economic Co-operation and Development), 2020. Test Guideline No. 471: Bacterial Reverse Mutation Test. OECD Guidelines for the Testing of Chemicals, Section 4.

Abbreviations

AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
CAS	Chemical Abstract Service
CBPI	Cytokinesis-Block Proliferation Index
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DMSO	dimethyl sulfoxide
EFFA	European Flavour Association
FAF	Panel on Food Additives and Flavourings
FEMA	Flavour and Extract Manufacturer Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
ID	identity
IR	infrared spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MN	micronuclei
MS	mass spectra
MSDI	Maximised 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 Organization

Appendix A – Specification Summary of the Substances in the Flavouring Group Evaluation 217Rev3

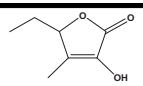
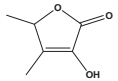
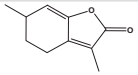
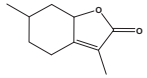
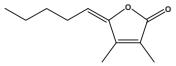
Table A.1: Specification Summary of the Substances in FGE.217Rev3 (JECFA, 2000, 2003, 2016b)

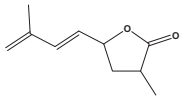
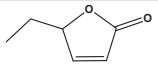
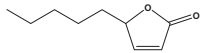
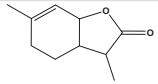
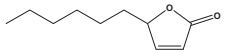
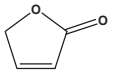
FL-no JECFA-no	Chemical name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]
10.023 222	5-Ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one		3153 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(5*H*)-one		3634 11834 28664-35-9	Liquid $C_6H_8O_3$ 128.13	Not reported by JECFA	81 (8 hPa) 25 IR 97.5%	Not reported by JECFA
10.057 2223	3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one		4140 57743-63-2	Liquid $C_{10}H_{14}O_2$ 166.10	Practically insoluble or insoluble Freely soluble	231–232 13 MS IR NMR > 95% (mixture of isomers) Isomeric composition: 81–84% (3a*S*,7a*R*); 16–19% (3a*R*,7a*S*).	1.490–1.496 1.065–1.071
13.012 1172	6-Methylcoumarin		2699 579 92-48-8	Solid $C_{10}H_8O_2$ 160.17	Insoluble Soluble	n.a. 73–79 IR 99%	Not reported by JECFA

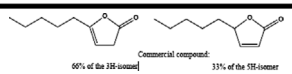
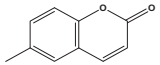
FL-no: FLAVIS number; FLAVIS: Flavour Information System (database); JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; ID: Identity; IR: infrared; NMR: nuclear magnetic resonance; MS: mass spectra; n.a.: not applicable.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1,013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

Appendix B – Summary of Safety Evaluation by JECFA, applying the Procedure

Table B.1: Summary of Safety Evaluation Applying the Procedure (JECFA, 1999, 2004, 2008, 2011, 2016a)

FL-no JECFA-no	Chemical name	Structural formula	EU MSDI[(a)] US MSDI (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (genotoxicity)
10.023 222	5-Ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one		13 6.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	Evaluated in FGE.217Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
10.030 243	3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one		2.1 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	Evaluated in FGE.217Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA considerations needed.
10.034 1163	5,6-Dihydro-3,6-dimethylbenzofuran-2(4*H*)-one		1 0.01	Class III A3: Intake below threshold	d	Evaluated in FGE.217Rev2, additional genotoxicity data were required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.
10.036 1162	5,6,7,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4*H*)-one		8 1	Class III A3: Intake below threshold	d	Evaluated in FGE.217Rev2, additional genotoxicity were data required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.
10.042 2002	3,4-Dimethyl-5-pentylidenefuran-2(5*H*)-one		0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	Evaluated in FGE.217Rev2, additional genotoxicity were data required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.

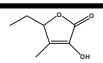
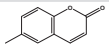
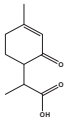
FL-no JECFA-no	Chemical name	Structural formula	EU MSDI[(a)] US MSDI (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (genotoxicity)
10.043	2,7-Dimethylocta-5 (trans),7-dieno-1,4-lactone		0.0012	No evaluation		Evaluated in FGE.217Rev2, additional genotoxicity data were required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.
10.046	Hex-2-eno-1,4-lactone		0.0024	No evaluation		Evaluated in FGE.217Rev2, additional genotoxicity data were required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.
10.054 2001	Non-2-eno-1,4-lactone		0.01	Class III A3: Intake below threshold	d	Evaluated in FGE.217Rev2, additional genotoxicity data were required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.
10.057 2223	3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one		0.012 300	Class III A3: Intake above the threshold, A4: metabolites not endogenous, A5: adequate NOAEL exists	d	Evaluated in FGE.217Rev3, no concern for genotoxicity. JECFA evaluated the substance based on SPET exposure assessment (300 μg/day)
10.060	2-Decen-1,4-lactone		0.037	Class III No evaluation		Evaluated in FGE.217Rev2, additional genotoxicity data were required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.
10.066 2000	Furan-2(5*H*)-one		0.01	Class III A3: Intake above the threshold, A4: metabolites not endogenous, A5: adequate NOAEL exists	d	Evaluated in FGE.217Rev2 as of genotoxicity concern.

FL-no JECFA-no	Chemical name	Structural formula	EU MSDI[(a)] US MSDI (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d) or (e)]	EFSA conclusion on the named compound (genotoxicity)
10.170 1989	5-Pentyl-3*H*-furan-2-one		1.2	Class II A3: Intake below threshold	d	Evaluated in FGE10Rev3, additional genotoxicity and specification data required. 1/3rd of the named substance correspond to [FL-no:10.054]. Evaluated in FGE.217Rev2, additional genotoxicity data required. No longer of interest for Industry for use as flavouring substance in Europe. No further data were submitted.
13.012 1172	6-Methylcoumarin		250 96	Class III B3: Intake above threshold; adequate data are available for the assessment	d	Evaluated in FGE.217. Genotoxicity concern could be ruled out. Evaluated using the Procedure in FGE.80Rev1: no safety concern at the estimated level of intake based on the MSDI approach.

FL-no: FLAVIS number; FLAVIS: Flavour Information System (database); JECFA: The Joint FAO/WHO Expert Committee on Food Additives; MSDI: Maximised Survey-derived Daily Intake; NOAEL: no-observed-adverse-effect-level; FGE: Flavouring Group Evaluation.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = μg/capita per day.
(b): Thresholds of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.

Appendix C – (Q)SAR Predictions on Mutagenicity

Table C.1: (Q)SAR Predictions on Mutagenicity for 3 Lactones from subgroup 4.1 and one precursor

FL-no JECFA-no	Subgroup	Chemical name	Structural formula[a]	FEMA no CoE no CAS no	ISS Local Model Ames Test TA100[b]	MultiCASE Ames test[c]	MultiCASE mouse lymphoma test[d]	MultiCASE chromosomal aberration test in CHO[e]	MultiCASE chromosomal aberration test in CHL[f]
10.023 222	4.1	5-Ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one		3153 2300 698-10-2	OD	NEG	NEG	NEG	NEG
10.030 243	4.1	3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one		3634 11834 28664-35-9	OD	NEG	NEG	NEG	NEG
13.012 1172	4.1	6-Methylcoumarin		2699 579 92-48-8	OD	POS	OD	OD	OD
Not in Register[4]	2.6	3-Methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one			OD	NEG	OD	NEG	EQU

(Q)SAR: (Quantitative) Structure–Activity Relationship; FL-no: FLAVIS number; FLAVIS: Flavour Information System (database); JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; CHO: Chinese hamster ovary (cells); CHL: Chinese hamster lung (cells); OD: Out of domain (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.); POS: Positive; NEG: Negative; EQU: Equivocal.
(a): Structural formula of substances in FGE.19 subgroup 4.1.
(b): Local model on aldehydes and ketones, Ames TA100.
(c): MultiCASE Ames test.
(d): MultiCASE mouse lymphoma test.
(e): MultiCASE chromosomal aberration in CHO.
(f): MultiCASE chromosomal aberration in CHL.

Appendix D – Genotoxicity data on 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one [FL-no: 10.057]

Table D.1: Summary of *in vitro* genotoxicity data on 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one [FL-no: 10.057]

Chemical name [FL-no]	Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reliability/comments	Reference
3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3*H*)-one [FL-no: 10.057]	Bacterial Reverse Mutation test	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. Coli* WP2 uvrA	156–5,000 μg/plate (pre-incubation method, without S9-mix, all bacterial strains) 39–1,250 μg/plate (pre-incubation method, with S9-mix, all *S. typhimurium* strains) 39–5,000 μg/plate (pre-incubation method, with S9-mix, *E. coli* WP2 uvrA)	Negative	Reliable without restrictions. Study performed according to OECD TG 471 and in compliance with GLP.	Documentation provided to EFSA No. 3
	Micronucleus assay	Human peripheral blood lymphocytes	700, 864 and 1,241 μg/mL (4 h + 20 h with S9-mix) 463, 813 and 1,239 μg/mL (4 h + 20 h without S9-mix) 40, 94 and 234 μg/mL (24 h without S9-mix)	Negative	Reliable without restrictions. Study performed according to OECD TG 487 and in compliance with GLP. The given concentrations are those for the cultures that were scored for micronuclei.	Documentation provided to EFSA No. 4