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

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SCIENTIFIC OPINION

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[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate the genotoxic potential of 12 flavouring substances from subgroup 4.1 of FGE.19 in the Flavouring Group Evaluation 217 (FGE.217). In FGE.217, 6-methylcoumarin [FL-no: 13.012] was not considered genotoxic and was therefore evaluated through the Procedure in FGE.80Rev1. For the remaining 11 substances, the Panel concluded that based on the data available, a genotoxic potential could not be excluded and accordingly they could not be evaluated through the Procedure. Additional data on genotoxicity for the three representative substances, 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023], 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066], have now been provided. Based on the new data, the Panel concluded that 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] does not give rise to concern with respect to genotoxicity and can accordingly, together with the structurally related substance, 3-hydroxy-4,5-dimethylfuran-2(5H)-one [FL-no: 10.030] for which it is a representative, be evaluated using the Procedure. For 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066] the concern for genotoxicity could not be ruled out and a combined micronucleus and Comet assay is 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].

KEY WORDS

FGE.217, alpha,beta-Unsaturated ketones, lactones, flavouring substances, safety evaluation, Subgroup 4.1, FGE.19

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[1] On request from the European Commission, Question No EFSA-Q-2013-00114, EFSA-Q-2013-00115, EFSA-Q-2013-00116, EFSA-Q-2013-00117, EFSA-Q-2013-00118, FSA-Q-2013-00119, EFSA-Q-2013-00120, EFSA-Q-2013-00121, EFSA-Q-2013-00122, EFSA-Q-2013-00287, EFSA-Q-2013-00288, adopted on 4 July 2013.

[2] Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Claudia Bolognesi, Leon Brimer, Laurence Castle, Alessandro Di Domenico, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Martine Kolf-Clauw, Catherine Leclercq, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Maria de Fatima Tavares Poças, Fidel Toldra and Detlef Wölfle. Correspondence: cef@efsa.europa.eu

[3] Acknowledgement : The Panel wishes to thank the members of the Genotoxicity Working Group on Flavourings: Mona-Lise Binderup, Wilfried Bursch, Angelo Carere, Riccardo Crebelli, Rainer Gürtler, Daniel Marzin, Pasquale Mosesso, for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft, Pia Lund, Karin Nørby and EFSA staff: Maria Carfi, Annamaria Rossi and Kim Rygaard Nielsen for the support provided to this scientific opinion.

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SUMMARY

Following a request from the European Commission the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate 12 flavouring substances in Flavouring Group Evaluation 217 (FGE.217) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000.

The FGE.217 concerned 12 substances, corresponding to subgroup 4.1 of FGE.19. The 12 substances are α,β-unsaturated lactones [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.043, 10.046, 10.054, 10.057, 10.060, 10.066 and 13.012], which by hydrolysis and oxidation gives rise to α,β-unsaturated ketones, which is a structural alert for genotoxicity.

In FGE.217, 6-methylcoumarin [FL-no: 13.012] was not considered genotoxic and was therefore allocated to FGE.80Rev1 for evaluation through the Procedure. For the remaining 11 substances, the Panel concluded that based on the data available, a genotoxic potential could not be excluded and accordingly they could not be evaluated through the Procedure. Additional data on genotoxicity for three representative substances, 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023], 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066], of this subgroup, should be provided. The present revision of FGE.217 (FGE.217Rev1) deals with additional data submitted by the Industry in response to the EFSA request expressed in FGE.217.

In vitro data in bacteria and mammalian test systems have now been provided for the three representative substances [FL-no: 10.023, 10.042 and 10.066] selected by the EFSA.

Based on these new data the Panel concluded that the genotoxic concern could be ruled out for 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] and accordingly this substance, and the one structurally related substance, 3-hydroxy-4,5-dimethylfuran-2(5H)-one [FL-no: 10.030], for which it is a representative, can be evaluated using the Procedure. For the two remaining representative substances, 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066], the test results from the studies in mammalian test systems raise concern with respect to genotoxicity *in vitro* and accordingly, these two substances [FL-no: 10.042 and 10.066] and the seven substances [FL-no: 10.034, 10.036, 10.043, 10.046, 10.054, 10.057 and 10.060] for which these two substance were representatives cannot be evaluated using the Procedure until additional *in vivo* genotoxicity data will become available. According to the recommendations of EFSA Scientific Committee (EFSA, 2011) a combined micronucleus and Comet assay should be considered. The Comet assay should be performed at least in the liver.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 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. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000.

EFSA has evaluated 12 flavouring substances, which correspond to subgroup 4.1 of FGE.19, in its evaluation of the flavouring group 217 (FGE.217). The opinion was adopted on 29 January 2009.

EFSA concluded that a genotoxic potential of the 11 α,β-unsaturated ketones and precursors in the present FGE.217 could not be ruled out.

Information on the three representative materials 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023], 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066] has now been submitted by the European Flavour Association. This information is intended to cover also the re-evaluation of the following eight substances from FGE.19 subgroup 4.1 (FGE.217):

- 3-Hydroxy-4,5-dimethylfuran-2(5H)-one [FL-no: 10.030]
- 5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one [FL-no: 10.034]
- 5,6,7,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4H)-one [FL-no: 10.036]
- 2,7-Dimethylocta-5(trans),7-dieno-1,4-lactone [FL-no: 10.043]
- Hex-2-eno-1,4-lactone [FL-no: 10.046]
- Non-2-eno-1,4-lactone [FL-no: 10.054]
- 3a,4.5.7a-Tetrahydro-3,6-dimethylbenzofuran-2(3H)-one [FL-no: 10.057]
- 2-Decen-1,4-lactone [FL-no: 10.060]

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substances.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the following eleven flavouring substances: 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023], 3-hydroxy-4,5-dimethylfuran-2(5H)-one [FL-no: 10.030], 5,6-dihydro-3,6-dimethylbenzofuran-2(4H)-one [FL-no: 10.034], 5,6,7,7a-tetrahydro-3,6-dimethylbenzofuran-2(4H)-one [FL-no: 10.036], 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042], 2,7-dimethylocta-5(trans),7-dieno-1,4-lactone [FL-no: 10.043], hex-2-eno-1,4-lactone [FL-no: 10.046], non-2-eno-1,4-lactone [FL-no: 10.054], 3a,4.5.7a-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one [FL-no: 10.057], 2-decen-1,4-lactone [FL-no: 10.060] and furan-2(5H)-one [FL-no: 10.066] in accordance with Commission Regulation (EC) N° 1565/2000.

HISTORY

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, 1999), as last amended by Commission Decision 2009/163/EC (EC, 2009). 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, 2000), 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, 2002).

The Union list of flavourings and source materials is established in Commission Regulation (EC) No 872/2012 (EC, 2012).

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

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

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

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

The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni and Netzeva, 2007a; Benigni and Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. 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) of FGE.19 (EFSA, 2008a) 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.

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 has worked out a list of

representative substances for each subgroup (EFSA, 2008c). Likewise, an EFSA genotoxicity expert group has worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008b).

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

The Flavouring Industry has now submitted additional data and the present revision of FGE.217 concerns the evaluation of these data requested on genotoxicity.

PRESENTATION OF THE SUBSTANCES BELONGING TO THE FLAVOURING GROUP EVALUATION 217 CORRESPONDING TO FGE.19 SUBGROUP 4.1

The 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, 2008a). All the substances are α,β-unsaturated lactones [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.043, 10.046, 10.054, 10.057, 10.060, 10.066 and 13.012], which by hydrolysis and oxidation give rise to alpha,beta-unsaturated ketones.

The α,β-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity (EFSA, 2008a). Accordingly, the available data on genotoxic or carcinogenic activity for the 12 lactones [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.043, 10.046, 10.054, 10.057, 10.060, 10.066 and 13.012] in FGE.217, anticipated to be metabolised to α,β-unsaturated ketones, will be considered in this FGE.

SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 217REV1

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

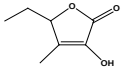
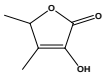
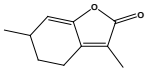
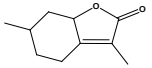
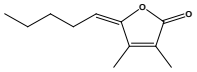
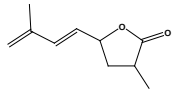
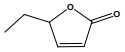
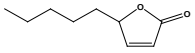
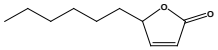
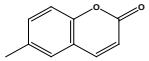
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
10.023 222	5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one		3153 2300 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		4050 11873 774-64-1	Liquid $C_{11}H_{16}O_2$ 180	Soluble Freely soluble	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 Freely soluble	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	Soluble	93 (13 hPa) 95 %	1.431-1.437 1.067-1.073
10.054	Non-2-eno-1,4-lactone		4188 21963-26-8	Liquid $C_9H_{14}O_2$ 154.21	Practically insoluble or insoluble Freely soluble	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 Freely soluble	231 13 95 %	1.494-1.500 1.053-1.059

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

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
10.060	2-Decen-1,4-lactone		 2518-53-8	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble Freely soluble	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 Freely soluble	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.

ASSESSMENT

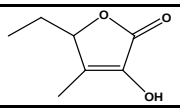
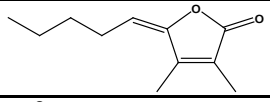
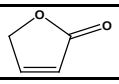
1. History of the FGE.217 Evaluation

In the first scientific opinion on FGE.217 (EFSA, 2009), the Panel concluded that additional genotoxicity data were required for 11 of the 12 α,β-unsaturated lactones considered in the FGE. For one substance, 6-methylcoumarin [FL-no: 13.012], the concern for genotoxicity could be ruled out and accordingly the substance could be evaluated using the Procedure in FGE.80Rev1. As 6-methylcoumarin is the only substance in FGE.217 with the α,β-ketone grouping in conjugation with an aromatic ring, this substance would not be considered a representative for 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 have been selected (Table 2) for the remaining 11 substances of FGE.19, subgroup 4.1, corresponding to FGE.217. 5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] is a representative for the structurally related substance 3-hydroxy-4,5-dimethylfuran-2(5H)-one [FL-no: 10.030], while 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no 10.042] and furan-2(5H)-one [FL-no 10.066] are representatives of the remaining seven substances [FL-no: 10.034, 10.036, 10.043, 10.046, 10.054, 10.057 and 10.060].

Table 2: Representative substances selected by EFSA for FGE.19 Subgroup 4.1 (FGE.217)

Representative substances for subgroup 4.1b of FGE.19 (EFSA, 2008c)

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

The Panel viewed the previous JEFCA evaluations (JECFA, 1998; JECFA, 2004) (Table 3) and reached the conclusions based on the data available at that time. These included a (Q)SAR prediction analysis (Table 4), a carcinogenicity study on 6-methylcoumarin [FL-no: 13.012] (Table 5), four *in vitro* studies (Table 6) and three *in vivo* studies on 6-methylcoumarin [FL-no: 13.012] (Table 7).

In Table 4 the outcomes of the (Q)SAR predictions for possible genotoxic activity in five *in vitro* (Q)SAR models (ISS Local Model-Ames test, DTU-NFI MultiCASE-Ames test, Chromosomal aberration test in Chinese hamster ovary 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].

The Carcinogenicity Study (Hagan et al., 1967) performed with 6-methylcoumarin [FL-no: 13.012] is reported in Table 5. 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. The NOAEL was 250 mg/kg bw/day based on growth depression and slight liver changes, particularly in males at the higher dose levels. No carcinogenicity was observed in this study (Hagan et al., 1967). The Panel also noted that this study was performed before OECD test guidelines 451/453 (1981) (OECD, 2009a and OECD, 2009b) 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 [FL-no: 13.012] was not carcinogenic in rats under the study conditions.

Genotoxicity studies were only available for 6-methylcoumarin [FL-no: 13.012]. In the Ames studies, 6-methylcoumarin was found negative in two valid tests (Haworth et al., 1983; Brusick, 1982), while results were equivocal in a valid study with strain TA100 (Wild et al., 1983) (Table 6). 6-Methylcoumarin was found negative in a valid mouse lymphoma tk assay (Cifone, 1982) (Table 6). Furthermore, 6-methylcoumarin was found negative in the 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. (Witt et al., 2000) (Table 7).

The Panel concluded that the data available do not indicate a genotoxic or carcinogenic potential for 6-methylcoumarin [FL-no: 13.012]. However, 6-methylcoumarin is the only substance in FGE.217 with the α,β-ketone grouping in conjugation with an aromatic ring, therefore, this substance would not be considered a representative for the remaining α,β-unsaturated lactones in this group.

Based on the data previously available, a genotoxic potential of the remaining 11 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] could not be excluded. Therefore, the Panel concluded that 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, 2008b).

FGE	Adopted by EFSA	Link	No. of Substances
FGE.217	29 January 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1068.htm	12
FGE.217Rev1	4 July 2013		12

2. Additional Genotoxicity Data Submitted for FGE 19, subgroup 4.1

Based on Panel request described in Section 1, additional data have been provided by Industry (IOFI, 2012a; IOFI, 2012b) for the three representative substances, 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023], dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066] (Table 2, Section 1), as requested by the EFSA. The present FGE.217, Revision 1 (FGE.217Rev1), includes the assessment of these additional genotoxicity data. The study types provided are shown below:

Substance / study type	Ames test	Micronucleus assay
5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023]	Bowen, 2011a	Lloyd, 2011
3,4-Dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042]	Bowen, 2011b	Whitwell, 2012a
Furan-2(5H)-one [FL-no: 10.066]	Bowen, 2011c	Whitwell, 2012b

2.1. *In vitro* data

2.1.1. Bacterial Reverse Mutation Assay

5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023]

5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] was tested for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9-mix), in two separate experiments. An initial toxicity range finding experiment was carried out in the absence and in the presence of the S9-mix in strain TA100 (Bowen, 2011a).

In experiment 1, treatments were performed in all tester strains in the absence and in the presence of S9-mix at concentrations of 1.6, 8, 40, 200, 1000 and 5000 µg/plate. Following these treatments, evidence of toxicity was observed in strain TA1537 in the presence of S9-mix at 5000 µg/plate and in strain TA102 in the presence of S9-mix at 200 µg/plate and above. Further evidence of toxicity in the form of a reduction in revertant numbers was observed in strain TA1535 in the presence of S9-mix and in strain TA102 in the absence of S9-mix at 5000 µg/plate.

In experiment 2, treatments were performed in all the tester strains in the absence and in the presence of S9-mix, using more narrow concentration intervals covering the range 156.3 - 5000 µg/plate. In addition, all treatments in the presence of S9-mix were further modified by the inclusion of a pre-incubation step. The maximum test concentration of 5000 µg/plate was retained for all strains. Following these treatments, evidence of toxicity was observed in the presence of S9-mix in strains TA1537 and TA102 at 2500 µg/plate and above. Further evidence of toxicity in the form of a reduction in revertant numbers was observed in strains TA98 in the presence of S9-mix at 5000 µg/plate and in strains TA98 and TA102 in the absence of S9-mix at 5000 and 2500 µg/plate, respectively.

No statistically significant increases in revertant numbers were observed following 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one treatments in any of the test strains, either in the absence or presence of S9-mix, in either experiment.

The Panel concluded that 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate, in the absence and in the presence of a rat liver metabolic activation system (S9-mix).

3,4-Dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042]

3,4-Dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] was tested for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9-mix), in two separate experiments and a third experiment performed in TA1537 (Bowen, 2011b).

In experiment 1, treatments were performed in all tester strains in the absence and in the presence of S9-mix at concentrations of 0.32, 1.6, 8, 40, 200, 1000 and 5000 µg/plate. Following these treatments evidence of toxicity was observed in all strains at the highest, second highest, and/or third highest concentrations in both the presence and absence of S9-mix metabolic activation.

In experiment 2, treatments were performed in all the tester strains in the absence and in the presence of S9-mix, using more narrow concentration intervals. For strains TA98, TA1535 and TA102, the range in both the absence and presence of S9-mix was 78.13 - 5000 µg/plate. For strain TA100 the concentration ranges were 78.13 - 5000 µg/plate in the presence of S9-mix and 19.53 - 1250 µg/plate in the absence of S9-mix. For strain TA1537 the concentration ranges were 9.76 - 1250 µg/plate in the absence of S9-mix and 78.13 - 5000 µg/plate in the presence of S9-mix. In this experiment all treatments done in the presence of S9-mix utilised a pre-incubation step. After incubation, evidence of toxicity was observed for all strains at 312.5 or 625 µg/plate and higher, except for strain TA102 in the presence of S9-mix where the toxicity was only observed at 1250 µg/plate and above. No increases in revertant numbers were observed in any strains in the presence or absence of S9-mix. For strain TA1537, there were too few non-toxic concentrations to fully assess the mutagenic potential in the presence of S9-mix. Therefore, a third experiment in the presence of S9-mix was carried out using the pre-incubation methodology at a concentration range of 19.53 - 1250 µg/plate. Evidence of toxicity was observed at 156.3 µg/plate and above. Thus, the study design complied with current recommendations from OECD Test Guideline 471 (OECD, 1997). No statistically significant increases in revertant numbers were observed.

The Panel concluded that 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium* when tested under the conditions of this study. These conditions included treatments up to toxic concentrations, in the absence and in the presence of a rat liver metabolic activation system (S9-mix).

Furan-2(5H)-one [FL-no: 10.066]

Furan-2(5H)-one [FL-no: 10.066] was tested for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S9-mix), in two separate experiments (Bowen, 2011c).

In experiment 1, treatments were performed in all tester strains in the absence and in the presence of S9-mix at concentrations of 0.32, 1.6, 8, 40, 200, 1000 and 5000 µg/plate. Following these treatments evidence of toxicity was observed in all strains at 5000 µg/plate with the exception of TA100 in the presence of S9-mix activation and TA1535 in the absence of S9-mix. No increases in revertant numbers were observed in any strains in the presence or absence of S9-mix.

In experiment 2, treatments were performed in all the tester strains in the absence and in the presence of S9-mix, using a narrower concentration range of 156.3 - 5000 µg/plate. In this experiment all treatments done in the presence of S9-mix utilized a pre-incubation step. Evidence of toxicity was observed for all strains in the presence and absence of S9-mix at 2500 and/or 5000 µg/plate. Thus, the study design complied with current recommendations from OECD Test Guideline 471 (OECD, 1997). No increases in revertant numbers were observed in any strains in the presence or absence of S9-mix.

The Panel concluded that furan-2(5H)-one [FL-no: 10.066] did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium* when tested under the conditions of this study. These conditions included treatments up to toxic concentrations, in the absence and in the presence of a rat liver metabolic activation system (S9-mix).

2.1.2. Micronucleus Assays

5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023]

5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] was tested for the induction of chromosome damage and potential aneugenic effects in an *in vitro* micronucleus assay using duplicate human peripheral blood lymphocytes prepared from pooled blood from two healthy male volunteers in

a single experiment. Treatments were performed both in the absence and presence of Aroclor 1254 induced rat liver S9-mix (Lloyd, 2011).

Treatment with 5-methyl-2-thiophenecarbaldehyde was conducted 48 hours after culture initiation (stimulation by phytohaemagglutinin).

A preliminary toxicity range finding experiment was conducted with and without S9-mix for 3 hours treatment and 21 hours of recovery (3 + 21 hours) and without S9-mix for 24 hours treatment. Toxicity was evaluated as the effect of treatment on the Replication Index (RI). Ten concentrations from 14,33 to 1422 µg/mL were tested. The concentrations selected for the main experiments were based on toxicity data from this preliminary test.

5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one was tested (Lloyd, 2011) at concentrations 1000, 1200 and 1422 µg/mL (equivalent to 10 mM), in the absence and presence of S9-mix, resulted in frequencies of micronucleated binucleate cells (MNBN), which were similar to those observed in concurrent vehicle controls for all concentrations analysed, and fell within historical vehicle control (normal) ranges. The above treatment concentrations induced maximum cytotoxicity (reduction in replication index) of 10 % in the absence of S9-mix activation and 23 % in the presence of S9-mix activation. Thus, the study design complies with current recommendations (including OECD Test Guideline 487 (OECD, 2010)). No increases in MNBN cells were observed following continuous 24 hours treatment in the absence of S9-mix at concentrations of 500, 750 and 900 µg/mL, the top concentration inducing 53 % cytotoxicity. These data indicated absence of induction of MNBN cells as a result of treatment with 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one at concentrations either reaching 10 mM or inducing 50 - 60 % toxicity.

The Panel concluded that 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] does not induce micronuclei in cultured human peripheral blood lymphocytes following treatment in the absence or in the presence of S9-mix. All values were within historical vehicle control ranges in all parts of the study and were not significantly different from concurrent controls.

3,4-Dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042]

3,4-Dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] was tested for the induction of chromosome damage and potential aneugenic effects in an *in vitro* micronucleus assay using duplicate human peripheral blood lymphocytes prepared from pooled blood from two healthy female volunteers in a single experiment. Treatments were performed both in the absence and presence of Aroclor 1254 induced rat liver S9-mix (Whitwell, 2012a).

A preliminary toxicity range finding experiment was conducted with and without S9-mix for 3 hours treatment followed by 21 hours recovery period and without S9-mix for 24 hours treatment. Toxicity was evaluated as the effect of treatment on the Replication Index (RI). Twelve concentrations from 7.256 to 2000 µg/mL were tested. The concentrations selected for the main experiments were based on toxicity data from this preliminary test (Whitwell, 2012a).

Cells were stimulated for 48 hours with phytohaemagglutinin to produce exponentially growing cells, and then treated for 3 hours (followed by 21 hours recovery) with 0, 40, 60, 70 and 90 µg/ml of 3,4-dimethyl-5-pentylidenefuran-2(5H)-one in the absence of S9-mix and 0, 60, 90, 110 and 140 µg/ml in the presence of S9-mix. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 57 % and 56 %, respectively. In a parallel assay, cells were treated for 24 hours with 0, 10, 13 and 15 µg/ml of 3,4-dimethyl-5-pentylidenefuran-2(5H)-one in the absence of S9-mix with no recovery period. The top concentration induced 57 % cytotoxicity. There were 2 replicate cultures per treatment, and 100 binucleate cells per replicate were scored for micronuclei. Thus the study design complies with current recommendations (OECD Test Guideline 487 (OECD, 2010)).

Treatment of cells with 3,4-dimethyl-5-pentylidenefuran-2(5H)-one for 3 hours with a 21 hours recovery period showed an increase in the frequency of MNBN cells at concentration levels of 70 and 90 µg/ml ($p \leq 0.05$) in the absence of S9-mix, but these were significantly below the 95 % confidence interval of the normal control range (0.10 - 1.60 %) and are not considered biologically relevant by the applicant. In the presence of S9-mix, treatment of cells with 3,4-dimethyl-5-pentylidenefuran-2(5H)-one for 3 + 21 hours showed an increase in the frequency of MNBN cells at concentration levels of 60 ($p \leq 0.01$), 90, 110 and 120 µg/ml ($p \leq 0.001$). No significant increases in MNBN frequencies were observed at any concentration after treatment for 24 hours with no recovery period. It was concluded that 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] induced micronuclei when assayed in cultured human peripheral lymphocytes for 3 + 21 hours in the presence of S9-mix (Whitwell, 2012a).

Furan-2(5H)-one [FL-no: 10.066]

Furan-2(5H)-one [FL-no: 10.066] was tested for the induction of chromosome damage and potential aneugenic effects in an *in vitro* micronucleus assay using duplicate human peripheral blood lymphocytes prepared from pooled blood from two healthy male volunteers in a single experiment. Treatments were performed both in the absence and presence of Aroclor 1254 induced rat liver S9-mix (Whitwell, 2012b).

A preliminary toxicity range finding experiment was conducted with and without S9-mix for 3 hours treatment and 21 hours recovery (3 + 21 hours) and without S9-mix for 24 hours treatment. Toxicity was evaluated as the effect of treatment on the Replication Index (RI). Twelve concentrations from 3.047 to 840 µg/mL were tested. The concentrations selected for the main experiments were based on toxicity data from this preliminary test (Whitwell, 2012b).

Cells were stimulated for 48 hours with phytohaemagglutinin to produce exponentially growing cells, and then treated for 3 + 21 hours with 0, 200, 350, 425, 450 and 475 µg/ml furan-2(5H)-one in the absence of S9-mix and 0, 100, 250, 425, 450 and 475 µg/ml in the presence of S9-mix. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 53 and 51 % respectively. In a parallel assay, cells were treated for 24 hours with 0, 10, 50, 60, 67.5 and 72.5 µg/ml of furan-2(5H)-one in the absence of S9-mix with no recovery period. The top concentration induced 61 % cytotoxicity. There were two replicate cultures per treatment, and 1000 binucleate cells per replicate were scored for micronuclei. Thus, the study design complies with current recommendations (OECD Test Guideline 487 (OECD, 2010)).

Treatment of cells with furan-2(5H)-one for 3 + 21 hours showed an increase in the frequency of MNBN cells at a concentration of 450 µg/ml ($p \leq 0.05$) in the absence of S9-mix, but it was associated with 64 % cytotoxicity to the cells and is not considered biologically relevant by the applicant. In the presence of S9-mix, treatment of cells with furan-2(5H)-one for 3 + 21 hours showed an increase in the frequency of MNBN cells at the three top concentrations ($p \leq 0.001$), and all were significantly above the 95 % confidence interval of the normal control range (0.10 - 1.10 %). Treatment for 24 hours with no recovery period showed an increase in MNBN frequencies at the top dose only, but it was lower than the 95 % confidence interval of the historical control range and it was associated with high cytotoxicity (61 %) by the applicant.

The Panel concluded that furan-2(5H)-one induces micronuclei when assayed in cultured human peripheral lymphocytes for 3 + 21 hours in the presence of S9-mix (Whitwell, 2012b).

The results of the additional *in vitro* studies are summarised in Table 8.

2.2. Additional available data

In more recent literature, the only reference to the potential of furanone compounds to induce DNA damage has been reported in association with the reduction of trivalent copper in an *in vitro* DNA damage assay (Murakami et al., 2007). Of three furanone analogues tested, 2,5-furanone (furaneol, 4-

hydroxy-2,5-dimethyl-furan-3-one [FL-no: 13.010] in FGE.220), 4,5-furanone (3-hydroxy-4,5-dimethylfuran-2(5H)-one [FL-no: 10.030]) and cyclotene (2-hydroxy-3-methyl-2-cyclopenten-1-one [07.056] in FGE.213 – not a furanone), only the first produced 8-hydroxy-2'-deoxyguanosine in DNA and strand breaks. These were associated with the generation of reactive oxygen species (superoxide radical) through the reduction of trivalent cupric to divalent cuprous ions. In contrast, to 2,5-furanone, the 4,5-analogue [FL-no: 10.030], which is one of the 12 substances evaluated in this group, did not produce a similar effect. These observations indicate that genotoxicity associated with members of the nine substances in group 4.1 is likely to be indirect and mediated via oxidative stress.

3. Conclusion

The FGE.217 concerned 12 substances, corresponding to subgroup 4.1 of FGE.19. The 12 substances are α,β-unsaturated lactones [FL-no: 10.023, 10.030, 10.034, 10.036, 10.042, 10.043, 10.046, 10.054, 10.057, 10.060, 10.066 and 13.012], which by hydrolysis and oxidation gives rise to α,β-unsaturated ketones, which is a structural alert for genotoxicity.

In FGE.217, 6-methylcoumarin [FL-no: 13.012] was not considered genotoxic and was therefore allocated to FGE.80Rev1 for evaluation through the Procedure. For the remaining 11 substances, the Panel concluded that based on the data available, a genotoxic potential could not be excluded and accordingly they could not be evaluated through the Procedure. Additional data on genotoxicity for three representative substances, 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023], 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066], of this subgroup, should be provided according to the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19. The present revision of FGE.217 (FGE.217Rev1) deals with additional data submitted by the Industry in response to the EFSA request expressed in FGE.217.

In vitro data in bacteria and mammalian test systems have now been provided for the three representative substances [FL-no: 10.023, 10.042 and 10.066] selected by the EFSA.

The three representative substances 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023], 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066] did not induce mutations in bacterial reverse mutation assays. In an *in vitro* micronucleus (MNvit) assay, 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] also did not reveal genotoxic effects under all test conditions according to OECD Test Guideline 487 (OECD, 2010). The Panel therefore concluded that the genotoxic concern could be ruled out for 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [FL-no: 10.023] and accordingly this substance and the one structurally related substance, 3-hydroxy-4,5-dimethylfuran-2(5H)-one [FL-no: 10.030] for which it is a representative, can be evaluated using the Procedure.

In the *in vitro* micronucleus assay 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] was negative in the 24 + 0 hour protocol, but equivocal results were obtained with 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] as well as for furan-2(5H)-one [FL-no: 10.066] in the 3 + 21 hours protocol in the absence of the S9-mix. Furthermore, in the presence of the S9-mix these two substances unequivocally induced micronuclei. The Panel therefore concluded that 3,4-dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042] and furan-2(5H)-one [FL-no: 10.066] raise concern with respect to genotoxicity *in vitro* and accordingly, these two substances [FL-no: 10.042 and 10.066] and the seven substances [FL-no: 10.034, 10.036, 10.043, 10.046, 10.054, 10.057 and 10.060] of subgroup 4.1 for which these two substance were representatives cannot be evaluated using the Procedure until additional *in vivo* genotoxicity data will become available. According to the recommendations of EFSA Scientific Committee (EFSA, 2011) a combined micronucleus and Comet assay should be considered. The Comet assay should be performed at least in the liver.

CURRENT SAFETY EVALUATION STATUS APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 3: Summary of Safety Evaluation of the JECFA substances in the present group (JECFA, 1998; JECFA, 2004)

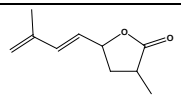
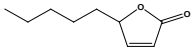
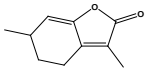
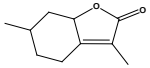
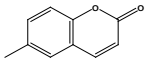
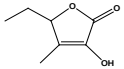
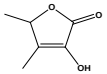
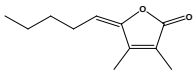
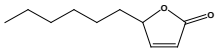
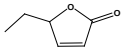
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	JECFA Outcome on the named compound [4] or 5)]	EFSA conclusion on the named compound (genotoxicity)
10.043	2,7-Dimethylocta-5(trans),7-dieno-1,4-lactone		0.0012	Class I No evaluation	Not evaluated by JECFA	Evaluated in FGE.217Rev1, additional genotoxicity data required.
10.054	Non-2-eno-1,4-lactone		0.012	Class I No evaluation	Not evaluated by JECFA	Evaluated in FGE.217Rev1, additional genotoxicity data required.
10.066	Furan-2(5H)-one		0.61	Class I No evaluation	Not evaluated by JECFA	Evaluated in FGE.217Rev1, additional genotoxicity data required.
10.034 1163	5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one		1.5 9	Class III A3: Intake below threshold	4)	Evaluated in FGE.217Rev1, additional genotoxicity data required.
10.036 1162	5,6,7,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4H)-one		3.5 9	Class III A3: Intake below threshold	4)	Evaluated in FGE.217Rev1, additional genotoxicity data required.
13.012 1172	6-Methylcoumarin		250 96	Class III B3: Intake above threshold	Data must be available 5)	Adequate data are available to reach the conclusion "No safety concern at the estimated level of intake based on the MSDI approach.".
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)	Evaluated in FGE.217Rev1, genotoxicity concern ruled out. Evaluated by JECFA before 2000.
.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)	Evaluated in FGE.217Rev1, genotoxicity concern ruled out. Evaluated by JECFA before 2000.
10.042	3,4-Dimethyl-5-pentylidenefuran-2(5H)-one		0.12	Class III No evaluation	Not evaluated by JECFA	Evaluated in FGE.217Rev1, additional genotoxicity data required.
10.060	2-Decen-1,4-lactone		0.037	Class III No evaluation	Not evaluated by JECFA	Evaluated in FGE.217Rev1, additional genotoxicity data required.

Table 3: Summary of Safety Evaluation of the JECFA substances in the present group (JECFA, 1998; JECFA, 2004)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	JECFA Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (genotoxicity)
10.046	Hex-2-eno-1,4-lactone		0.0024	No evaluation	Not evaluated by JECFA	Evaluated in FGE.217Rev1, additional genotoxicity data required.
10.057	3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3H)-one		0.012	No evaluation	Not evaluated by JECFA	Evaluated in FGE.217Rev1, additional genotoxicity data required.

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.

QSAR PREDICTIONS ON MUTAGENICITY IN FIVE MODELS FOR 10 LACTONES FROM SUBGROUP 4.1

Table 4: QSAR Predictions on Mutagenicity in Five Models for 10 Lactones from Subgroup 4.1 and two precursors

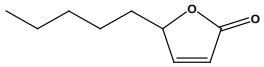
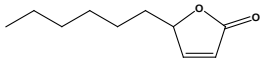
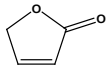
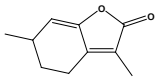
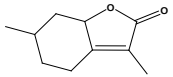
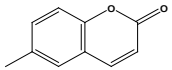
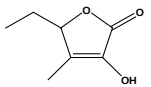
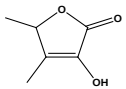
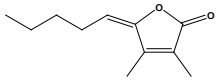
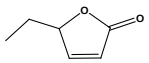
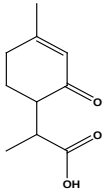
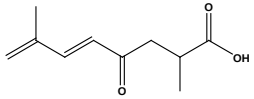
FL-no JECFA-no	Sub-group	EU Register name	Structural formula	FEMA no CoE no CAS no	ISS Local Model Ames Test TA100	MultiCASE Ames test	MultiCASE Mouse lymphoma test	MultiCASE Chromosomal aberration test in CHO	MultiCASE Chromosomal aberration test in CHL
10.054	4.1	Non-2-eno-1,4-lactone		4188 - 21963-26-8	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

Table 4: QSAR Predictions on Mutagenicity in Five Models for 10 Lactones from Subgroup 4.1 and two precursors

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
10.046	4.1	Hex-2-eno-1,4-lactone		- - 2407-43-4	OD	NEG	OD	POS	OD
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.

CARCINOGENICITY STUDIES

Table 5: 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.

GENOTOXICITY (*IN VITRO*)

Table 6: 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, 1982)	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, 1982)	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, 1982)	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).

GENOTOXICITY (*IN VIVO*)

Table 7: 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).

NEW GENOTOXICITY (*IN VITRO*)

Table 8: Summary of Additionally Genotoxicity Data [FL-no: 10.023, 10.042 and 10.066] of subgroup 4.1

Chemical Name [FL-no:]	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one [10.023]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	1.6, 8, 40, 200, 1000 and 5000 µg/plate [1,2]	Negative	(Bowen, 2011a)	Valid study in accordance with OECD Guideline 471 (OECD, 1997) and in compliance with GLP. Evidence of toxicity was observed in strain TA1537 in the presence of S9-mix at 5000 µg/plate and in strain TA102 in the presence of S9-mix at 200 µg/plate and above. Further evidence of toxicity in the form of a reduction in revertant numbers was observed in strain TA1535 in the presence of S9-mix and in strain TA102 in the absence of S9-mix at 5000 µg/plate.
		S. typhimurium TA98, TA100, TA1535, TA1537 and TA102	156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate [2,3]	Negative		Evidence of toxicity was observed in the presence of S9-mix in strains TA1537 and TA102 at 2500 µg/plate and above. Further evidence of toxicity in the form of a reduction in revertant numbers was observed in strains TA98 in the presence of S9-mix at 5000 µg/plate and in strains TA98 and TA 102 in the absence of S9-mix at 5000 and 2500 µg/plate, resp.
			156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate [4,5]	Negative		
	Micronucleus Assay	Human peripheral blood lymphocytes	1000, 1200 and 1422 µg/ml (equivalent to 10 mM) [1,6]	Negative	(Lloyd, 2011)	Valid study in accordance with draft OECD Guideline 487 (OECD, 2010) and in compliance with GLP. A top concentration of 10 mM was employed or an acceptable level of cytotoxicity was achieved at the top concentration used in the continuous treatment schedule.
			500, 750 and 900 µg/ml [3,7]	Negative		
3,4-Dimethyl-5-pentylidenefuran-2(5H)-one [10.042]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA102	0.32, 1.6, 8, 40, 200, 1000 and 5000 µg/plate [1,2]	Negative	(Bowen, 2011b)	Valid study in accordance with OECD Guideline 471 (OECD, 1997) and in compliance with GLP. Evidence of toxicity was observed in all strains in the absence and presence of S9 at 200 µg/plate and above.
		S. typhimurium TA98, TA102, TA1535	78.13 - 5000 µg/plate [2,3] 78.13 - 5000 µg/plate [4,5]	Negative		Evidence of toxicity was observed in strain TA102 in the presence of S9 at 1250 µg/plate and above, and for all other treatment conditions at either 312.5 or 625 µg/plate and above.
		S. typhimurium TA100	19.53 - 1250 µg/plate [2,3] 78.13 - 5000 µg/plate [4,5]			
		S. typhimurium TA1537	9.76 - 1250 µg/plate [2,3] 78.13 - 5000 µg/plate [4,5]			
		S. typhimurium TA1537	19.53 - 1250 µg/plate [4,5]	Negative		Evidence of toxicity was observed at 156.3 µg/plate and above.

Table 8: Summary of Additionally Genotoxicity Data [FL-no: 10.023, 10.042 and 10.066] of subgroup 4.1

Chemical Name [FL-no:]	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
	In vitro Micronucleus induction	Human peripheral blood lymphocytes	40, 60, 70 and 90 µg/ml [3,6]	Equivocal	(Whitwell, 2012a) Whitwell (2012a)	Valid study in accordance with OECD Guideline 487 (OECD, 2010) and in compliance with GLP.
			60, 90, 110, and 140 µg/ml [5,6]	Positive		
			10, 13, and 15 µg/ml [3,7]	Negative		
Furan-2(5H)-one [10.066]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	0.32, 1.6, 8, 40, 200, 1000, and 5000 µg/plate [1,2]	Negative	(Bowen, 2011c)	Valid study in accordance with OECD Guideline 471 (OECD, 1997) and in compliance with GLP. Evidence of toxicity was observed in all treatment conditions in the absence and presence of S9 at 5000 µg/plate, with the exception of TA100 in the presence of S9 and strain TA1535 in the absence of S9.
			156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate [2,3] 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate [4,5]	Negative		Evidence of toxicity was observed in all treatment conditions in the absence and presence of S9 at 2500 and/or 5000 µg/plate.
	In vitro Micronucleus induction	Human peripheral blood lymphocytes	200, 350, 425, 450 and 475 µg/ml [3,6]	Equivocal	(Whitwell, 2012b)	Valid study in accordance with OECD Guideline 487 (OECD, 2010) and in compliance with GLP.
			100, 250, 425, 450 and 475 µg/ml [5,6]	Positive		
			10, 50, 60, 67.5 and 72.5 µg/ml [3,7]	Equivocal		

[1] With and without S9 metabolic activation.
[2] Plate incorporation method.
[3] Without S9 metabolic activation.
[4] Pre-incubation method.
[5] With S9 metabolic activation.
[6] 3-hour incubation with 21-hour recovery period.
[7] 24-hour incubation with no recovery period.

REFERENCES

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.

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.

Bowen R, 2011a. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. 5-Ethyl-3-hydroxy-4-methyl-2(5H)furanone. Covance Laboratories Ltd. Study no. 8226869. January 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bowen R, 2011b. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. 3,4-Dimethyl-5-pentylidenefuran-2(5H)-one. Covance Laboratories Ltd. Study no. 8233097. October 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bowen R, 2011c. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. Furan-2(5H)-one. Covance Laboratories Ltd. Study no. 8233099. September 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Brusick DJ, 1982. Mutagenicity evaluation of 6-methylcoumarin in the Ames salmonella/microsome plate test. Revised final report. Litton Bionetics. LBI project no. 20988. June, 1982. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Cifone MA, 1982. Mutagenicity evaluation of 6-methylcoumarin in the mouse lymphona forward assay. Final report. Litton Bionetics. LBI project no. 20989. October, 1982. Unpublished report submitted by EFFA to FLAVIS Secretariat.

EC (European Commission), 1996. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC (European Commission), 1999. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC (European Commission), 2000. Commission Regulation 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. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EC (European Commission), 2002. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

EC (European Commission), 2009. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.

EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. Official Journal of the European Communities 2.10.2012, L 267, 1-161.

EFSA AFC Panel (EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food) 2008a. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. Available online: http://www.efsa.europa.eu/EFSA/Event_Meeting/afc_minutes_26thplen_en.pdf

EFSA (European Food Safety Authority), 2008b. Genotoxicity Test Strategy for Substances belonging to Subgroups of FGE.19. The EFSA Journal 2008, 854, 1-5.

EFSA (European Food Safety Authority), 2008c. List of alpha, beta-unsaturated aldehydes and ketones representative of FGE.19 substances for genotoxicity testing. The EFSA Journal 2008, 910, 1-5.

EFSA (European Food Safety Authority), 2009. Flavouring Group Evaluation 217: alpha,beta-Unsaturated ketones and precursors from chemical subgroup 4.1 of FGE.19: Lactones. The EFSA Journal 2009, 1068, 1-20.

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. doi:10.2903/j.efsa.2011.2379.

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.

Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM, Long EL, Nelson AA and Brouwer JB, 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food and Cosmetics Toxicology 5(2), 141-157.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environmental Mutagenesis 5(Suppl. 1), 3-142.

IOFI (International Organization of the Flavor Industry), 2012a. Flavouring Group Evaluation 19 Subgroup 4.1a: 9 Flavouring Substances (Flavouring Substances) of the Chemical Group 3 (Annex I of 1565/2000/EC) Heterocyclic alfa,beta-unsaturated aldehydes, ketones and related substances with the alfa,beta-conjugation in the ring or in the side chain, Lactones Used as Flavouring Substances. 27/11/2012. FLAVIS/8.171.

IOFI (International Organization of the Flavor Industry), 2012b. Flavouring Group Evaluation 19 Subgroup 4.1b: 15. Flavouring Substances (Flavouring Substances) of the Chemical Group 3 (Annex I of 1565/2000/EC) Heterocyclic alfa,beta-unsaturated aldehydes, ketones and related substances with the alfa,beta-conjugation in the ring or in the side chain, Lactones Used as Flavouring Substances. 17/12-2012. FLAVIS/8.181.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1998. 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), 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.

Lloyd M, 2011. Induction of micronuclei in cultured human peripheral blood lymphocytes. 5-Ethyl-3-hydroxy-4-methyl-2(5H)furanone. Unaudited draft report. Covance Laboratories LTD. Study no. 8226870. January 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Murakami K, Haneda M, Makino T and Yoshino M, 2007. Prooxidant action of furanone compounds: Implication of reactive oxygen species in the metal-dependent strand breaks and the formation of 8-hydroxy-2'-deoxyguanosine in DNA. Food and Chemical Toxicology 45, 1258-1262.

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.

OECD (Organisation for Economic Co-operation and Development), 1997. OECD guideline for testing of chemicals. No. 471: Bacterial Reverse Mutation Test. Adopted on 21st July 1997.

OECD (Organisation for Economic Co-operation and Development), 2009a. OECD guideline for testing of chemicals. No. 451: Carcinogenicity Studies. Adopted on 7th September 2009.

OECD (Organisation for Economic Co-operation and Development), 2009b. OECD guideline for testing of chemicals. No. 453: Combined Chronic Toxicity\Carcinogenicity Studies. Adopted on 7th September 2009.

OECD (Organisation for Economic Co-operation and Development), 2010. OECD guideline for testing of chemicals. No. 487: In Vitro Mammalian Cell Micronucleus Test. Adopted on 22nd July 2010.

SCF (Scientific Committee on Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Whitwell J, 2012a. Induction of micronuclei in cultured human peripheral blood lymphocytes. 3,4-Dimethyl-5-pentylidenefuran-2(5H)-one. Covance Laboratories Ltd, England. Study no.8233098. April 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Whitwell J, 2012b. Induction of micronuclei in cultured human peripheral blood lymphocytes. Furan-2(5H)-one. Covance Laboratories Ltd, England. Study no.8233100. February 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Wild D, King MT, Gocke E and Eckhard K, 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food and Chemical Toxicology 21(6), 707-719.

Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD and MacGregor JT, 2000. Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. Environmental and Molecular Mutagenesis 36(3), 163-194.

ABBREVIATIONS

BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese Hamster Ovary (cells)
CHL	Chinese Hamster Lung (cells)
CoE	Council of Europe
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
ID	Identity
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	MicroNucleated BiNucleate cells
MS	Masse Spectra
NMR	Nuclear Magnetic Resonance
No	Number
NAOEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PCE	PolyChromatic Erythrocytes
(Q)SAR	(Quantitative) Structure Activity Relationship
RI	Replication Index
SCF	Scientific Committee on Food
WHO	World Health Organisation