Scientific Opinion on Flavouring Group Evaluation 220 Revision 3 (FGE.220Rev3): Consideration of genotoxic potential for ,-unsaturated 3(2H)-Furanones from subgroup 4.4 of FGE.19

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 220 Revision 3 (FGE.220Rev3): Consideration of genotoxic potential for α,β-unsaturated 3(2H)-Furanones from subgroup 4.4 of FGE.19 [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 10 flavouring substances from FGE.19 subgroup 4.4, in the Flavouring Group Evaluation 220 (FGE.220). FGE.220 is subdivided into two subgroups: subgroup 4.4a containing [FL-no: 13.089, 13.117, 13.119, 13.157 and 13.175] and subgroup 4.4b containing [13.010, 13.084 and 13.085, 13.099 and 13.176]. For both subgroups the Panel concluded that the genotoxicity alert could not be ruled out based on the data available and accordingly additional genotoxicity data were requested. In FGE.220, Revision 1, the Panel concluded that for the substances in subgroup 4.4b there is no concern for genotoxicity. In FGE.220, Revision 2, the Panel evaluated genotoxicity studies on two representative substances of subgroup 4.4a: 2,5-dimethylfuran-3(2H)-one [FL-no:13.119] and 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175]. Based on the submitted data the Panel concluded that 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119] does not give rise to concern with respect to genotoxicity. For 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] the concern for genotoxicity could not be ruled out, therefore the Panel requested a repetition of the submitted micronucleus study in the presence of S9-mix, or a combined *in vivo* micronucleus and Comet assay, including analysis of the liver. The Flavour Industry has tested again 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] in an *in vitro* micronucleus assay as was requested by the Panel. This new study is evaluated in FGE.220, Revision 3, where the Panel concluded that [FL-no: 13.175] does not give rise to concern with respect to genotoxicity and can be evaluated using the Procedure. This is also applicable to other two substances in subgroup 4.4a: 2,5-dimethyl-4-methoxyfuran-3(2H)-one [FL-no:13.089] and 2,5-dimethyl4-ethoxyfuran-3(2H)-one [FL-no:13.117]. Based on the available data the substances of this FGE are no longer of concern with respect to genotoxicity and can be evaluated through the Procedure.

[1] On request from the European Commission, Question Nos EFSA-Q-2014-00684, EFSA-Q-2014-00685 and EFSA-Q-2014-00686, adopted on 7 May 2015.

[2] Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Konrad Grob, Rainer Gürtler, Trine Husøy, Wim Mennes, Maria Rosaria Milana, André Penninks, Vittorio Silano, Andrew Smith, Maria de Fátima Tavares Poças, Christina Tlustos, Fidel Toldrá, Detlef Wölfle and Holger Zorn. Correspondence: fip@efsa.europa.eu

[3] The Panel wishes to thank the members of the Genotoxicity Working Group on Flavourings: Mona-Lise Binderup, Claudia Bolognesi, Riccardo Crebelli, Rainer Gürtler, Natália Kovalkovičová, Francesca Marcon, Daniel Marzin and Pasquale Mosesso for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby, and EFSA staff: Annamaria Rossi and Maria Carfi for the support provided to this scientific opinion.

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KEY WORDS

α,β-unsaturated ketones, 3(2H)-furanones, flavouring substances, safety evaluation, FGE.220, FGE.19, subgroup 4.4

SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate 10 flavouring substances in Flavouring Group Evaluation 220 (FGE.220) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000.

Flavouring Group Evaluation 220 (FGE.220) concerned 10 substances, corresponding to subgroup 4.4 of FGE.19. The 10 substances are α,β-unsaturated 3(2H)-furanones [FL-no: 13.010, 13.084, 13.085,13.089, 13.099, 13.117, 13.119, 13.157, 13.175 and 13.176]. The substances were further subdivided into two subgroups as five [FL-no: 13.089, 13.117, 13.119, 13.157 and 13.175] of the 10 substances can only exist as α,β-unsaturated ketones (subgroup 4.4a) while in the other five substances [13.010,13.084 and 13.085, 13.099 and 13.176], the α,β-double bond can be involved in keto-enol tautomerism (subgroup 4.4b). For both groups the Panel concluded that the genotoxicity alert could not be ruled out based on data available at that time, and accordingly additional genotoxicity data were requested for both groups.

Revision 1 of FGE.220 (FGE.220Rev1) concerned the evaluation of additional data submitted by Industry in response to the requested genotoxicity data in FGE.220 on the representative substance for subgroup 4.4b, 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010]. The Panel concluded that for the substances [13.010, 13.084 and 13.085, 13.099 and 13.176] in subgroup 4.4b there is no concern for genotoxicity, and these substances were accordingly evaluated through the Procedure in FGE.99.

In FGE.220, Revision 2, genotoxicity data related to subgroup 4.4a were evaluated. The Flavour Industry informed that one of the representative substances, 5-methylfuran-3(2H)-one [FL-no: 13.157], is not in common use in the Flavour Industry and is no longer supported. As an alternative substance for testing within this subgroup, the Flavour Industry had proposed the structurally related substance 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119]. Accordingly, additional genotoxicity data were submitted for 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175]. These data were examined by the Panel which concluded that 2,5-dimethylfuran-3(2H)-one [FLno:13.119] does not give rise to concern with respect to genotoxicity and can accordingly be evaluated using the Procedure. For 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] the concern for genotoxicity could not be ruled out and therefore the Panel requested a repetition of the micronucleus study in the presence of S9-mix, applying the same conditions and possibly in addition modified conditions, or a combined *in vivo* micronucleus and Comet assay, including analysis of liver.

The Industry has tested again 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] in an *in vitro* micronucleus assay in the presence of S9-mix (3+21 hours). These data are evaluated in the present Revision 3 of FGE.220 (FGE.220 Rev3). The available data suggest that 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] did not induce reproducible statistically significant increase in micronucleated binucleate cells across replicate cultures indicating that the test substance can be considered negative for clastogenicity and aneugenicity. The Panel therefore concluded that [FL-no: 13.175] does not give rise to concern with respect to genotoxicity and accordingly can be evaluated using the Procedure. This is also applicable to 2,5-dimethyl-4-methoxyfuran-3(2H)-one [FL-no:13.089] and 2,5-dimethyl4-ethoxyfuran-3(2H)-one [FL-no:13.117] which are covered by the representative substance 4-acetyl2,5-dimethylfuran-3(2H)-one [FL-no:13.175].

Based on the available data all 9 substances of this FGE are no longer of concern with respect to genotoxicity and can be evaluated through the Procedure.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

On 25 September 2013, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 220 (FGE.220Rev2): Consideration of genotoxicity data on representatives for heterocyclic α,β-unsaturated Aldehydes, Ketones and Related Substances with the α,β-conjugation in the Ring or in the side chain.

On the basis of the data supplied, the Opinion concluded that the concern for genotoxicity could not be ruled out for the substances in this subgroup and therefore the Panel request a repetition of micronucleus study in the presence of S9-mix applying the same conditions and possible in addition modified conditions, or by a combined *in vivo* micronucleus study and Comet assay, including analysis of the liver.

The applicant has submitted additional data in response to this EFSA evaluation.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to evaluate this new information and, depending on the outcome, proceed to the full evaluation on these flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p.34–50.

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

[6] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8–16.

METHODOLOGIES AND DATA

Methodologies

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

The α,β-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity (EFSA, 2008a). The Panel noted that there were limited genotoxicity data on these flavouring substances but that positive genotoxicity studies were identified for some substances in the group.

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

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

The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni and Netzeva, 2007a; Benigni and Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. Based on these data the Panel decided that 15 subgroups (1.1.1, 1.2.1, 1.2.2, 1.2.3, 2.1, 2.2, 2.3, 2.5, 3.2, 4.3, 4.5, 4.6, 5.1, 5.2 and 5.3) (EFSA, 2008a) could not be evaluated through the Procedure due to concern with respect to genotoxicity. Corresponding to these subgroups, 15 Flavouring Group Evaluations (FGEs) were established: FGE.200, 204, 205, 206, 207, 208, 209, 211, 215, 219, 221, 222, 223, 224 and 225.

For 11 subgroups the Panel decided, based on the available genotoxicity data and (Q)SAR predictions, that a further scrutiny of the data should take place before requesting additional data from the Flavouring Industry on genotoxicity. These subgroups were evaluated in FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220. For the substances in FGE.202, 214 and 218 it was concluded that a genotoxic potential could be ruled out and accordingly these substances will be evaluated using the Procedure. For all or some of the substances in the remaining FGEs, FGE.201, 203, 210, 212, 213, 216, 217 and 220 the genotoxic potential could not be ruled out.

To ease the data retrieval of the large number of structurally related α,β-unsaturated substances in the different subgroups for which additional data are requested, EFSA worked out a list of representative substances for each subgroup (EFSA, 2008c). Likewise an EFSA genotoxicity expert group has worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008b).

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

Data

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

ASSESSMENT

1. History of the Evaluation of the Substances in Subgroup 4.4

Subgroup 4.4 of FGE.19 consists of ten α,β-unsaturated 3(2H)-furanones, which have been further subdivided into two groups 4.4a and 4.4b based on chemical structures (Table 2). For both groups the Panel concluded that the genotoxicity alert could not be ruled out based on data available at that time, and accordingly additional genotoxicity data were requested for both groups. The additional information should be based on specific data requested in FGE.220 and performed on representative substances selected from both groups (EFSA, 2008c).

In the EFSA Opinion "List of α,β-unsaturated aldehydes and ketones representative of FGE.19 substances for genotoxicity testing" (EFSA, 2008c), representative flavouring substances have been selected for subgroups 4.4a and 4.4b, corresponding to FGE.220, for which additional data on genotoxicity were requested, according to the Opinion of the Panel on the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19" (EFSA, 2008b).

Revision 1 of FGE.220 (FGE.220Rev1), concerns the evaluation of additional data submitted by Industry in response to the requested genotoxicity data in FGE.220 on the representative substance for subgroup 4.4b, 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010]. These new data are described and evaluated in Section 4 in the present version of FGE.220.

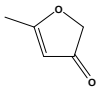
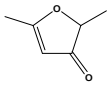
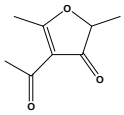
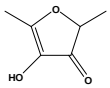
Revision 2 of FGE.220 (FGE.220Rev2), concerns the evaluation of additional data submitted by Industry in response to requested genotoxicity data in FGE.220 on representative substances for subgroup 4.4a (see Table 1). The Flavour Industry has informed that one of the representative substances, 5-methylfuran-3(2H)-one [FL-no: 13.157], is not in common use in the flavour industry and is no longer supported. As an alternative substance for testing within this subgroup, the Flavour Industry had proposed the structurally related substance of subgroup 4.4a, 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119] and submitted data on this substance and on 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] (Table 1).

FGE	Adopted by EFSA	Link	No. of Substances
FGE.220	29 January 2009	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902503180.htm	10
FGE.220Rev1	30 September 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1841.htm	10
FGE.220Rev2	25 September 2013	http://www.efsa.europa.eu/en/efsajournal/pub/3390.htm	9
FGE.220Rev3	7 May 2015	http://www.efsa.europa.eu/en/efsajournal/pub/4117.htm	9

The present revision of FGE.220 concerns the evaluation of new data received for 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] (Lloyd, 2014). The new information supplied by the Industry is a repetition of the short-term treatment, (3+ 21hours) in the presence of S9 metabolism, of a micronucleus study earlier submitted and which showed equivocal results in the short-term treatment (3+21 hours) in the presence of S9-mix (Lloyd, 2012).

Table 1: Representative substances selected for FGE.19 Subgroup 4.4 (FGE.220)

Representative substances selected by EFSA for FGE.19 Subgroup 4.4 (FGE.220) (EFSA, 2008c)

Subgroup	FL-no	Register name for representatives	Structural formula
4.4a	13.157	5-Methylfuran-3(2H)-one Not supported any longer by EFFA	
	13.119	2,5-Dimethylfuran-3(2H)-one New representative substance suggested by EFFA	
	13.175	4-Acetyl-2,5-dimethylfuran-3(2H)-one	
4.4b	13.010	4-hydroxy-2,5-dimethylfuran-3(2H)-one	

In Section 6, the evaluation of the new genotoxicity data received for 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] is described.

Sections 2 - 5 report the same information that was presented in the earlier versions of FGE.220.

2. Presentation of the Substances in Flavouring Group Evaluation 220

The present Flavouring Group Evaluation 220 (FGE.220) concerns nine substances, which are presented in Table 2. The nine substances correspond to subgroup 4.4 of FGE.19 (EFSA, 2008a). These substances are all α,β-unsaturated 3(2H)-furanones [FL-no: 13.010, 13.084, 13.085, 13.089, 13.099, 13.117, 13.119, 13.175 and 13.176]. Four of the nine substances can only exist as ketones [FL-no: 13.089, 13.117, 13.119 and 13.175] (subgroup 4.4a). In the remaining five substances, the α,β-double bond can be involved in keto-enol tautomerism as such [FL-no: 13.010, 13.084 and 13.085] or after hydrolysis of the ester moiety [13.099 and 13.176] (subgroup 4.4b).

A summary of their current evaluation status of both subgroups 4.4a and 4.4b by the JECFA is given in Appendix A, Table 3 (JECFA, 2006).

The α,β-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008a). Accordingly, the available data on genotoxic or carcinogenic activity for the ten ketones in FGE.220 were considered.

The Panel has also taken into consideration the outcome of the predictions from five selected (Q)SAR models (Benigni & Netzeva, 2007a; Gry et al., 2007; Nikolov et al., 2007) on the ketones in the present FGE. The ten α,β-unsaturated ketones and their (Q)SAR predictions are described in Section 3.1 and summarised in Appendix B, Table 4.

Table 2: Specification Summary of the Substances in the Present Group Evaluation (JECFA, 2002)

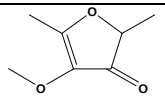
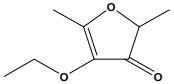
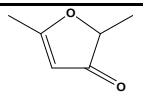
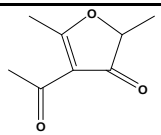
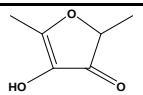
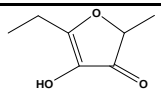
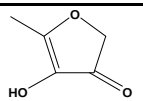
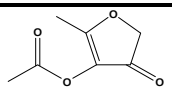
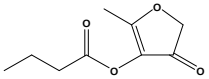
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec. gravity [e]
Substances in subgroup 4.4a (Furan-3(2H)-ones)							
13.089 1451	2,5-Dimethyl-4-methoxyfuran-3(2H)-one		3664 4077-47-8	Liquid $C_7H_{10}O_3$ 142.15	Insoluble Soluble	61-63 (0.4 hPa) - NMR 97 %	1.475-1.481 1.091-1.097
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2H)-one		 65330-49-6	Solid $C_8H_{12}O_3$ 156.18	Freely soluble	251 60 - 95 %	n.a. n.a.
13.119	2,5-Dimethylfuran-3(2H)-one		 11066 14400-67-0	Liquid $C_6H_8O_2$ 112.13	Practically insoluble Freely soluble	68 (16 hPa) - IR NMR MS 95 %	1.473-1.479 1.050-1.060
13.175	4-Acetyl-2,5-dimethylfuran-3(2H)-one			Solid $C_8H_{10}O_3$ 154.17	Freely soluble	283 34 - 95 %	n.a. n.a.
Substances in subgroup 4.4b (Furan-3(2H)-ones in which the alpha,beta-unsaturated double bond can be involved in keto-enol tautomerism)							
13.010 1446	4-Hydroxy-2,5-dimethylfuran-3(2H)-one		3174 536 3658-77-3	Solid $C_6H_8O_3$ 128.13	Insoluble Soluble	n.a. 78-80 IR NMR MS 98 %	n.a. n.a.
13.084 1449	2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone		3623 27538-09-6	Liquid $C_7H_{10}O_3$ 142.15	Soluble Soluble	103 (20 hPa) - IR NMR 96 %	1.509-1.514 1.133-1.143
13.085 1450	4-Hydroxy-5-methylfuran-3(2H)-one		3635 11785 19322-27-1	Solid $C_5H_6O_3$ 114.10	Soluble Soluble	n.a. 126-133 NMR 97 %	n.a. n.a.

Table 2: Specification Summary of the Substances in the Present Group Evaluation (JECFA, 2002)

FL-no JECFA-no	EU Register 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)]
13.099 1456	4-Acetoxy-2,5-dimethylfuran-3(2H)-one		3797 4166-20-5	Liquid $C_8H_{10}O_4$ 170.17	Slightly soluble Soluble	243 - IR NMR MS 95 %	1.476-1.480 1.159-1.167
13.176 1519	Furaneyl butyrate		3970	Liquid $C_{10}H_{14}O_4$ 198.22	Insoluble Soluble	287 - NMR 95 %	1.467-1.473 1.095-1.103

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.
n.a: not applicable.

3. Data available to and Evaluated by the Panel in FGE.220[7]

3.1. (Q)SAR Predictions

In Appendix B, 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 none of the candidate substances in this FGE a prediction was obtained with the ISS Local Model for gene mutations in *Salmonella* TA100, as all substances were out of domain. The DTU-NFI MultiCase models for mutagenicity predicted negative (no genotoxic potential) in the Ames test for all 10 substances, and also for three substances (all three in subgroup 4.4b) in the Mouse lymphoma assay. For one substance [FL-no: 13.157] from subgroup 4.4a, a positive response in the Mouse lymphoma assay was predicted. The other candidate substances were out of domain. All but four substances from the subgroup were out of domain for both the Chromosomal aberration CHO and CHL models. The four substances from subgroup 4.4b were in the domain of the Chromosomal aberrations CHL model and for these four the application of the model resulted in a negative prediction.

It is concluded that these models, except for the negative predictions for the substance in the DTU-NFI MultiCASE model for Ames test, do not seem to generate a reliable and reproducible pattern of predictions for this group. Negative predictions in mammalian cells were only available for four of the substances in subgroup 4.4b (furan-3(2H)-ones in which the α,β- double bond can be involved in keto-enol tautomerism). One positive prediction was available for genotoxic activity in mammalian cells for a substance in subgroup 4.4a (furan-3(2H)-ones).

3.2. Carcinogenicity Studies

A carcinogenicity study with chronic exposure is available for one substance in subgroup 4.4b.

In an OECD Guideline 451 and GLP compliant study, groups of 60 male and 60 female Sprague-Dawley rats were fed diets containing 0 (controls), 100, 200 or 400 mg 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] /kg body weight (bw) per day for two years. Mean body weights and body weight gains of male and female rats exposed to 400 mg 4-hydroxy-2,5-dimethyl-3(2H)-furanone/kg bw per day were decreased compared to those of the controls in the last part of the study. No neoplasms or non-neoplastic lesions were attributed to exposure to 4-hydroxy-5-dimethyl-3(2H)-furanone. The NOAEL was 200 mg/kg bw per day (Kelly and Bolte, 2003).

The Panel concluded that the study on 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] was valid and did not show a carcinogenic potential in rats.

Study validation and results are presented in Appendix C, Table 5.

3.3. Genotoxicity Studies

Genotoxicity studies are available for four of the candidate substances in FGE.220, as summarised in Appendix C, Tables 6 and 7.

Subgroup 4.4a (Furan-3(2H)-ones)

For one substance in subgroup 4.4a (2,5-dimethyl-3(2H)-furanone [FL-no: 13.119]) no mutagenic activity was observed in *S. typhimurium* in a valid assay. No experimental data were available for any of the other substances in this subgroup.

[7] The data presented in Section 3 are cited from the first version of the present FGE.220. These data are the basis of the conclusions in FGE.220 requesting additional genotoxicity data.

Subgroup 4.4b (Furan-3(2H)-ones in which the α,β- double bond can be involved in keto-enol tautomerism).

For the three remaining substances with available genotoxicity data, which belong to subgroup 4.4b the following results have been reported.

4-Hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010]

For 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] publications on *in vitro* and *in vivo* studies are available. In three studies the potential of the test substance to induce gene mutations in *S. typhimurium* was studied. The substance was found positive in two valid studies (Gilroy et al, 1978; Hiramoto et al, 1996b) and in one study with limited validity (Xing et al, 1988). The substance did not cause gene mutations in a valid study in *Escherichia coli* WP2 uvrA$^-$ (Gilroy et al, 1978). It was also observed that the substance caused DNA repair in a less relevant bacterial test (Xing et al, 1988) and single strand breaks in purified DNA (Hiramoto et al, 1996b).

All *in vivo* studies provided indications for a genotoxic potential. Two studies showing micronucleus formation in peripheral blood cells were considered valid (Hiramoto et al., 1996b; Hiramoto et al., 1998); in a third study similar evidence but of limited validity was obtained (Xing et al., 1988). The latter authors also reported an increase in sister chromatid exchanges (SCE) in mouse bone marrow, but the validity of that observation could not be assessed. In addition this endpoint is of questionable relevance for the assessment of genotoxicity.

In addition to the genotoxicity observed in somatic cells, three studies provided evidence for genotoxicity in germ cells.

The evidence of chromosome aberration induction in mouse germ cells provided in the study by Xing et al. (1988) is poor because it is essentially based on an increase of premature disjunction of sex chromosomes and autosomes at metaphase I. This effect could be considered at most an alert of possible subsequent missegregation events; even so, data have been published (Liang and Pacchierotti, 1988) showing the lack of correlation between univalents at metaphase I and aneuploidy at metaphase II.

Tian et al. (1992) reported an induction of SCE in spermatogonia. Incomplete information is given on the experimental protocol. There is a dose-dependent increase of SCE/cell, with each dose group significantly higher than the negative control. For these reasons, these data seem to be convincing although obtained on a small number (3) of animals/group. The relevance of SCE in spermatogonia as an indicator of heritable genetic damage is limited.

In the same paper Tian et al. (Tian et al., 1992) reported the induction of micronuclei in early sperm cells. This test measures the induction of DNA lesions in preleptotene spermatocytes that can lead to breaks and fragments several days later, at the first or second meiotic division. The test has not been standardised and validated for routine regulatory application, but has been conducted by more than one laboratory in the world with consistent results. The study seems adequately performed. Staining with Giemsa is not optimal and does not allow to distinguish among phases of spermatid differentiation as recommended by the guidelines (Russo, 2000). However, this drawback could hardly produce an overestimation of the effect, more likely, if any, an underestimation.

4-Hydroxy-5-methylfuran-3(2H)-one [FL-no: 13.085] and 2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084]

Reverse mutations were also observed in *S. typhimurium* TA100, but not TA98 with 4-hydroxy-5-methylfuran-3(2H)-one [FL-no: 13.085] (Hiramoto et al, 1996a) and with 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084] (Li et al, 1998). The other strains were not tested. The same substances could induce single strand breaks in purified DNA (Hiramoto et al, 1996a; Li et al, 1998).

With 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [FL-no: 13.084] also induction of micronuclei in peripheral erythrocytes was observed in two valid *in vivo* assays (Hiramoto et al, 1998; Li et al, 1998).

Mechanistic data

For the substances in subgroup 4.4b also mechanistic studies were carried out with [FL-no: 13.010, 13.084 and 13.085], all of which were considered valid. These substances were identified as Maillard reaction products in soy sauce. When the substance [FL-no: 13.085] was incubated with supercoiled pBR 322 plasmid DNA, single strand breaks were observed at pH 4.4, but not at pH 7.4. When a spin trap was also present, formation of hydroxy radicals together with a carbon-centered radical could be demonstrated. Subsequent addition of superoxide dismutase and catalase inhibited the DNA breaking showing involvement of hydrogen peroxide. Potassium iodide, mannitol, sodium azide and ethanol were also inhibitory to the DNA breaking showing involvement of hydroxy radicals. Spin trapping agents and thiol compounds and metal chelators also effectively inhibited the breaking of DNA (Hiramoto et al., 1996a). Similar studies (Hiramoto et al., 1996b; Li et al., 1998) were carried out with [FL-no: 13.010 and 13.084] with the same results and it was also demonstrated that these substances are capable to reduce Fe^{3+} at neutral or alkaline pH (Li et al., 1998).

Study results and comments on study validity are presented in Appendix C, Tables 6 and 7.

3.4. Conclusion on Genotoxicity and Carcinogenicity in FGE.220

Apart from the negative predictions for the substances in the DTU-NFI MultiCASE model for the Ames test, the (Q)SAR models do not seem to generate a reliable and reproducible pattern of predictions on the genotoxicity for the substances in this FGE.

For one substance in subgroup 4.4a (2,5-dimethyl-3(2H)-furanone [FL-no: 13.119]) no mutagenic activity was observed in *S. typhimurium* in a valid assay. This study result is insufficient to reach a conclusion as to the absence of genotoxicity for this subgroup.

With several substances in subgroup 4.4b indications have been obtained in *in vitro* studies that the genetic damage they cause is related to the generation of reactive oxygen species as a result of redox cycling in combination with metal ions present in the media. The valid positive *in vivo* data were obtained with high dose levels that may be anticipated to have exhausted the anti-oxidant capacity of the target cells. This, in combination with the absence of carcinogenicity observed in a valid carcinogenicity study in rats with one of the substances [FL-no: 13.010], which was tested positive in the genotoxicity assays, takes away a concern for genotoxic events resulting in carcinogenicity in somatic cells.

For two of the studies in which genotoxic effects were observed in germ cells *in vivo* the studies had limited validity and/or address endpoints that may have limited relevance for the assessment of genotoxic potential. The Panel noted that a positive result was obtained in a micronucleus study in early sperm cells. However, a micronucleus test does not discriminate between aneuploidy and chromosomal breakage. The observed effects in the germ cells could be the result of the malsegregation of chromosomes which is generally considered a thresholded event. They may alternatively be the result of the (thresholded) generation of reactive oxygen species.

3.5. Conclusions in FGE.220

For the substances in subgroup 4.4a [FL-no: 13.089, 13.117, 13.119, 13.157 and 13.175], the Panel considered that presently the available data on genotoxicity are too limited to evaluate these substances through the Procedure. Additional studies are needed as outlined in the Genotoxicity Test Strategy for Substances belonging to Subgroups of FGE.19 (EFSA, 2008b).

For the substances in subgroup 4.4b [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176], evidence for genotoxicity was obtained *in vitro* and *in vivo*. Evidence is available from *in vitro* studies that the

genotoxicity of the candidate substances in this subgroup may be caused by indirect (thresholded) mechanisms of action (in particular generation of reactive oxygen species). The concern for carcinogenicity is alleviated, since one of the substances, for which positive genotoxicity data in mice were obtained, was not carcinogenic in a valid chronic assay in rats. Therefore, no further genotoxicity tests in somatic cells are required. However, some evidence was also available that this substance might elicit genotoxic effects in germ cells, which theoretically may result in reduced reproductive capacity or in inheritable genetic damage. Reduced reproductive capacity and inheritable genetic damage are toxicological endpoints which differ from carcinogenicity and therefore, the negative results for the carcinogenicity study cannot be used to overrule this concern. Also it is not clear if (and if so to what extent) the thresholded mechanism mentioned above would be relevant for genotoxic effects in the germ cells. Therefore, the Panel concluded that presently these five substances cannot be evaluated through the Procedure.

The Panel recognised that the studies which provided indications for germ cell genotoxicity are of limited validity. For that reason a robust GLP-controlled cytogenetic investigation in mouse spermatocytes according to the OECD Guideline 483 is requested.

4. Additional Genotoxicity Data Evaluated by the Panel in FGE.220Rev1[8]

4.1. Evaluation of Additional Data for Subgroup 4.4b

In response to the EFSA request in FGE.220, of a cytogenetic study in mouse spermatocytes (OECD TG 483), Industry has submitted the following data:

- 2-year carcinogenicity bioassay in rats with a substance coded ST 07 C99 (this is the study on [FL-no: 13.010] by Kelly & Bolte, 2003);
- oral male fertility study of FURANEOL = 4-Hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] (test article code ST17C07) in rats (Sloter, 2008);
- oral micronucleus assay in bone marrow cells of the mouse with neofuraneol (no identification of this substance is available) (Honarvar, 2008);
- mouse lymphoma (TK) specific locus mutation assay with compound 0478/1 (Ross & Harris, 1979a).

The Panel noted that among the studies submitted by Industry only the rat fertility study, which includes also the analysis of dominant lethals, is considered relevant for the specific EFSA request.

The 2-year carcinogenicity bioassay in rats by Kelly and Bolte (Kelly and Bolte, 2003) was already evaluated by the Panel in the previous version of this FGE (Section 3.2 and Table 5). It was considered as a valid, negative study, however not relevant for the evaluation of possibly inheritable damage. Also the mouse bone marrow micronucleus assay with neofuraneol (Honarvar, 2008) and the *in vitro* mouse lymphoma TK assay (Ross and Harris, 1979) are considered not relevant to clear the concern for possible inheritable damage. Furthermore, an adequate identification of the test substance neofuraneol was not possible, due to incomplete reporting. For these reasons these three studies will not be further considered in this section.

Oral Male Fertility Study of 4-Hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] in Rats (Sloter, 2008)

The objective of this study, performed according to ICH Guideline 4.1.1 (ICH, 1996) under GLP, was to determine the potential effects of 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] on

[8] The data presented in Section 4 are cited from the revision 1 of FGE.220 (FGE.220Rev1). These data are the basis of the conclusions in FGE.220Rev1 requesting additional genotoxicity data.

mating, fertility and gonadal function in male rats with two separate mating trials. 4-Hydroxy-2,5-dimethylfuran-3(2H)-one was administered by gavage once daily to three groups of 25 male Crl:CD(SD) rats. Dosage levels were 100, 500 and 1000 mg/kg bw/day. A concurrent control group of 25 males received the vehicle (propylene glycol) on a comparable regimen. The first mating (Phase I), following 2 weeks of male administration, using untreated females, was conducted to detect potential elicitation of early genotoxic effects on the embryo with reduced risk of test-article related deficiencies in mating or fertility. The second mating (Phase II), following 9 weeks of male dose administration, was conducted following male exposure throughout a complete spermatogenic cycle using a second set of untreated females.

There was no test-article related mortality noted in this study. A slightly lower mean body-weight gain was noted in the 1000 mg/kg/day group when evaluated for the overall treatment period. No test-article related effects on male reproductive performance were observed at 100, 500 and 1000 mg/kg per day when males were mated with Phase I or Phase II females. In particular, there were no effects on spermatogenic endpoints (mean testicular and epididymal sperm numbers, sperm production rate, motility and morphology, reproductive organs or macroscopic findings) at any of the doses tested. The mean percentage of sperm with abnormal morphology (separated head and flagellum) was slightly higher in the 500 and 1000 mg/kg per day groups; however, this was primarily attributed to a single male in the respective groups and therefore not considered test-article related. The number of females mated and the number of pregnant females was comparable to controls. Uterine examination was performed for both Phase I and Phase II females. The analysis of embryonic data (corpora lutea, implantation sites, viable embryos, dead embryos, early resorptions, late resorptions, total resorptions, post- and pre-implantation losses) did not reveal dominant lethal effects. The study does not indicate a potential of 4-hydroxy-2,5-dimethylfuran-3(2H)-one [FL-no: 13.010] to affect male fertility. This study can be considered to be equivalent to an OECD 478 Dominant Lethal assay. The Dominant Lethal assay has been recommended as follow-up study in case of positive results in the OECD Guideline 483 (Eastmond et al., 2009). On this basis, the Panel considers it acceptable to substitute the requested study according to OECD Guideline 483 with the Dominant Lethal test.

Study results and comments on study validity are presented in Appendix C, Table 8.

4.2. Conclusion on Additional Data in FGE.220Rev1

The results of a valid rat fertility and dominant lethal study have shown that 4-hydroxy-2,5-dimethylfuran-3(2H)-one is unable to induce both adverse effects on male rat reproductive capacity and dominant lethality. On this basis, the Panel concludes that for this substance there is no concern for its potential to induce heritable genetic damage or adverse effects on male reproductive capacity. Accordingly the substances in subgroup 4.4b of FGE.19 [FL-no: 13.010, 13.084, 13.085, 13.099 and 13.176] can be evaluated using the Procedure. Since no data were submitted to further evaluate the genotoxic potential of the substances in subgroup 4.4a, the Panel in FGE.220Rev1 maintained its position that for this subgroup additional data on genotoxicity are needed.

5. Additional Genotoxicity Data Evaluated by the Panel in FGE.220Rev2[9]

5.1. *In vitro* Genotoxicity Studies for Subgroup 4.4a

In response to the EFSA request in FGE.220 for additional genotoxicity data for subgroup 4.4a the Flavour Industry (IOFI, 2012) has submitted *in vitro* genotoxicity data on:

- 2,5-Dimethylfuran-3(2H)-one [FL-no: 13.119] (Ames test and *in vitro* micronucleus assay)
- 4-Acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] (Ames test and *in vitro* micronucleus assay)

[9] The data presented in Section 5 are cited from the revision 2 of FGE.220 (FGE.220Rev2). These data are the basis of the conclusions in FGE.220Rev2 requesting additional genotoxicity data

Bacterial mutation assays

2,5-Dimethylfuran-3(2H)-one [FL-no: 13.119]

2,5-Dimethylfuran-3(2H)-one [FL-no: 13.119] was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence or presence of S9-mix (Sokolowski, 2007). In the first experiment the concentrations tested were 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate, and plate incorporation methodology was used. In the second experiment the concentrations were 33, 100, 333, 1000, 2500 and 5000 µg/plate of 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119], and treatments in the absence and in the presence of S9-mix used the pre-incubation method. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. The solvent control data reported for strain TA102 in the absence of S9-mix, indicated slightly increased numbers of revertant colony numbers (538 ± 28) compared to historical controls (407.1 ± 78.3). Since the effect is small in the control, the effect is considered by the study director to be based upon biologically irrelevant fluctuations in the number of colonies. Thus, the study design complied with current recommendations and an acceptable top concentration was achieved. There was no evidence of any mutagenic effect induced by 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119] in any of the strains, either in the absence or presence of S9-mix.

4-Acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175]

4-Acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] was tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 in the absence and presence of S9-mix (Bowen, 2011). In the first experiment the concentrations were 0.32, 1.6, 8, 40, 200, 1000 and 5000 µg/plate of 4-acetyl-2,5-dimethylfuran-3-(2H)-one [FL-no: 13.175] and the plate incorporation methodology was used. Slight thinning of the background lawn was observed at 5000 µg/plate for all test strains in the absence and presence of S9-mix. In the second experiment the concentrations were 78.13, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate and the treatments in the presence of S9-mix used the pre-incubation method. No clear evidence of toxicity was observed. Thus, the study design complied with current recommendations and an acceptable top concentration was achieved. There was no evidence of any mutagenic effect induced by 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] in any of the strains, either in the absence or presence of S9-mix.

Micronucleus Assays

2,5-Dimethylfuran-3(2H)-one [FL-no: 13.119]

2,5-Dimethylfuran-3(2H)-one [FL-no: 13.119] was evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9-mix fraction as an *in vitro* metabolising system. Cells were stimulated for 48 hours with phytohaemagglutinin (PHA) to produce exponentially growing cells, and then treated for 3 hours (followed by 21 hours recovery) with 0, 900, 1000 or 1120 µg/ml of 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119] in the absence and in the presence of S9-mix. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 12 and 2 % respectively. In a parallel assay, cells were treated for 24 hours with 0, 900, 1000 and 1120 µg/ml of 2,5-dimethylfuran-3(2H)-one [FL-no: 13.119] in the absence of S9-mix with no recovery period. The top concentration induced 22 % cytotoxicity. There were 2 replicate cultures per treatment, and 1000 binucleate cells per replicate (i.e. 2000 cells per concentration) were scored for micronuclei. Thus the study design complies with current recommendations (including draft OECD Guideline 487), and acceptable levels of cytotoxicity were achieved at the top concentrations used in all parts of the study. No evidence of chromosomal damage or aneuploidy was observed by increased levels of micronucleated binucleate cells (MNBN) in the presence or absence of S9-mix metabolic activation (Lloyd, 2011).

4-Acetyl-2,5-dimethylfuran-3(2H)-one [FL 13.175]

In a similar experiment, human peripheral lymphocyte cells were stimulated for 48 hours with PHA to produce exponentially growing cells, and then treated for 3 hours (followed by 21 hours recovery) with 0, 1000, 1250 or 1542 µg/ml of 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] in the absence and in the presence of S9-mix. The levels of cytotoxicity (reduction in replication index) at the top concentrations were 20 and 7 % respectively.

In a parallel assay, cells were treated for 24 hours with 0, 400, 600, 900 and 950 µg/ml of 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] in the absence of S9-mix with no recovery period. The top concentration induced 54 % 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 (including draft OECD guideline 487), and acceptable levels of cytotoxicity were achieved at the top concentrations used in all parts of the study.

Initially (following the scoring of 1000 binucleate cells/culture), treatment of cells for 3 hours with a 21 hour recovery period in the presence of S9-mix resulted in mean frequencies of MNBN cells (0.55% , 0.85 % and 1.25 %, at 1000, 1250 and 1542 µg/ml, respectively) that were significantly higher ($p \leq 0.01$) compared with those observed in concurrent controls (0.20 %) at all three concentrations analysed, giving 3 %, 0 % and 7 % reductions in RI, respectively. The MNBN cell frequencies exceeded the normal range (0.1 % to 1.1 %) only in single cultures at 1250 and 1542 µg/ml (1.2 % and 1.6 %, respectively). It was noted that one of the solvent control replicates fell to 0 %, which is outside of historical control levels and would have impacted the statistical significance.

To confirm this result additional 1000 binucleate cells were scored for the vehicle controls "C" and "D" replicate cultures derived from new human blood cultures and an additional 1000 binucleate cells were scored from each of the three test article concentrations analysed, derived from the same human blood culture used in the first experiment. Following the additional scoring, the mean frequencies of MNBN cells were significantly higher but at lower statistical level ($p \leq 0.05$), compared to those observed in the concurrent vehicle controls at the two highest concentrations analysed (1250 and 1542 µg/ml). It was noted that only one culture at 1542 µg/ml (1.25 %) exceeded the normal range.

The Panel noted that the additional scoring was conducted with an unjustified and non-homogeneous approach; while for the solvent controls the additional 1000 cells derived from new blood cultures, for the treated cells the additional scoring derived from the same blood cultures used in the first experiment. Overall, differently from the authors, the Panel concluded that the results of the *in vitro* micronucleus assay in the presence of S9-mix have to be considered as equivocal instead of negative and therefore the test should be repeated (Lloyd, 2012).

Study results and comments on study validity are presented in Appendix C, Table 9.

5.2. Conclusions on Additional Genotoxicity Data in FGE.220Rev2

2,5-Dimethylfuran-3(2H)-one [FL-no: 13.119] did not induce mutations in the Ames test and did not induce increased levels of micronuclei in an *in vitro* micronucleus assay with and without metabolic activation. The Panel therefore concluded that [FL-no: 13.119] does not give rise to concern with respect to genotoxicity and accordingly can be evaluated using the Procedure.

4-Acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] did not induce mutations in the Ames test with and without metabolic activation and did not induce increased levels of micronuclei in an *in vitro* micronucleus assay in the absence of S9-mix. However, the results of the micronucleus assay in the presence of S9-mix were considered by the Panel to be equivocal. Therefore, the results of the *in vitro* micronucleus assay should be clarified, e.g. by repetition of the study in the presence of S9-mix applying the same conditions and possibly in addition modified conditions, or by a combined *in vivo* micronucleus and comet assay, including analysis of liver. This is also applicable to 2,5-dimethyl-4-methoxyfuran-3(2H)-one [FL-no: 13.089] and 2,5-dimethyl-4-ethoxyfuran-3(2H)-one [FL-no: 13.117]

which are covered by the representative substance 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175].

6. Additional Genotoxicity Data Evaluated by the Panel in FGE.220Rev3

6.1. *In vitro* Micronucleus Study for [FL-no: 13.175]

In response to the request to clarify the results of an *in vitro* micronucleus assay, the Industry has submitted a new study with 4-acetyl-2,5-dimethylfuran-3(2H)-one: "Induction of micronuclei in cultured human peripheral blood lymphocytes (Lloyd, 2014)".

This study is a follow-up of the *in vitro* micronucleus study (Lloyd, 2012) evaluated in FGE.220Rev2. In that study (Lloyd, 2012) the induction of micronuclei in human lymphocytes treated with [FL-no: 13.175] for 3+21 hours, in the presence of S9-mix, was considered by the Panel to be equivocal, therefore a repetition of the experiment was requested.

The new study (Lloyd, 2014) was conducted to investigate the reproducibility of the findings under the test conditions of 3+21 hours in the presence of S9-mix. In the follow-up study, the frequency of micronuclei was assessed in cultured human peripheral blood lymphocytes (whole blood cultures pooled from two healthy male volunteers in a single experiment) following treatment with the same concentrations (1000, 1250 and 1542 µg/mL) of 4-acetyl-2,5-dimethylfuran-3(2*H*)-one as before in the presence of a metabolising system (S9-mix) from livers of rats induced with Aroclor 1254. After 48 hours of culture initiation (stimulation by PHA), cultures were treated for 3 hours followed by 21 hours of recovery. The highest concentration was equal to the highest concentration used in the previous study and is equivalent to 10 mM, or the maximum required test concentration (MW = 154.2). Cyclophosphamide (CPA 3.0 µg/mL) was used as a clastogenic positive control chemical in the presence of rat liver S9-mix. Cytochalasin B (6 µg/ml) was added at the end of the 3-hour treatment in order to block cytokinesis and generate binucleate cells for analysis, and it remained in the cultures during the recovery period.

In this follow-up study, unlike the findings of the previously reported study, a marginally significant increase ($p \leq 0.05$) in the mean frequency of micronuclei was reported only at the lowest of the three concentrations of 4-acetyl-2,5-dimethylfuran-3(2*H*)-one tested (1000 µg/mL) when compared to the concurrent vehicle control following scoring of 8000 cells (4000 cells per replicate). However, the MNBN frequencies of both replicate cultures (0.68 % and 0.5 %) at this concentration remained well within the normal historical control range (0.1 to 0.9 %). This finding shows that 4-acetyl-2,5-dimethylfuran-3(2*H*)-one did not induce reproducible and consistent increases of micronuclei frequency across replicate cultures in independent studies, indicating that the observed statistically significant increases in MNBN in the first study (Lloyd, 2012) are of no biological relevance. On this basis, 4-acetyl-2,5-dimethylfuran-3(2*H*)-one is considered negative for clastogenicity and aneugenicity in the *in vitro* micronucleus assay when tested up to a maximum concentration of 1542 µg/mL for 3 hours plus 21 hours recovery period in the presence of S9-mix.

Study results and comments on study validity are presented in Appendix C, Table 9.

6.2. Conclusions on Additional Genotoxicity Data in FGE.220Rev3

4-Acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] did not induce mutations in the Ames test with and without metabolic activation (Bowen, 2011). There was an equivocal result observed in an *in vitro* micronucleus assay, however, in the follow up study (Lloyd, 2014) 4-acetyl-2,5-dimethylfuran-3(2*H*)-one did not induce reproducible statistically significant increase in MNBN cells across replicate cultures indicating that the test substance can be considered negative for clastogenicity and aneugenicity. The Panel therefore concluded that 4-acetyl-2,5-dimethylfuran-3(2H)-one [FL-no: 13.175] does not give rise to concern with respect to genotoxicity and accordingly can be evaluated using the Procedure. These results are also applicable to two other substances in subgroup 4.4a: 2,5-

dimethyl-4-methoxyfuran-3(2H)-one [FL-no: 13.089] and 2,5-dimethyl-4-ethoxyfuran-3(2H)-one [FL-no: 13.117].

Based on the available data all 9 substances of this FGE are no longer of concern with respect to genotoxicity and can be evaluated through the Procedure.

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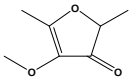
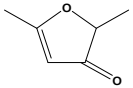
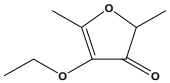
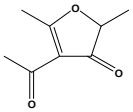
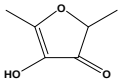
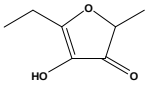
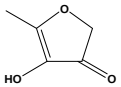
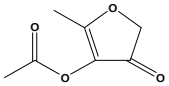
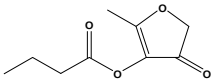
Appendix A. Summary of Safety Evaluation Applying the Procedure

Table 3: Summary of Safety Evaluation Applying the Procedure

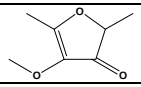
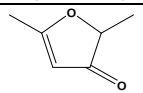
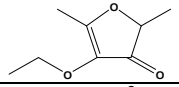
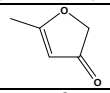
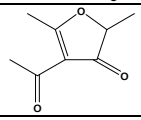
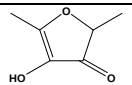
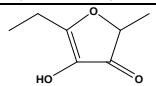
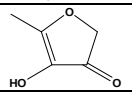
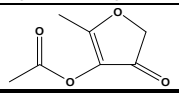
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (μg/*capita*/day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d), (e)]	EFSA conclusion on the named compound: (Procedure steps, intake estimates, NOAEL, genotoxicity)
Substances in subgroup 4.4a (Furan-3(2H)-ones)						
13.089 1451	2,5-Dimethyl-4-methoxyfuran-3(2H)-one		0 0.7	Class II A3: Intake below threshold	d	Evaluated in FGE.220Rev3, genotoxicity concern ruled out, can be evaluated using the Procedure.
13.119	2,5-Dimethylfuran-3(2H)-one		1.9	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required	Evaluated in FGE.220Rev2, genotoxicity concern ruled out, can be evaluated using the Procedure.
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2H)-one		0.018	No evaluation		Evaluated in FGE.220Rev3, genotoxicity concern ruled out, can be evaluated using the Procedure.
13.175	4-Acetyl-2,5-dimethylfuran-3(2H)-one		1.3	No evaluation		Evaluated in FGE.220Rev3, genotoxicity concern ruled out, can be evaluated using the Procedure.
Substances in subgroup 4.4b (Furan-3(2H)-ones in which the alpha,beta-unsaturated double bond can be involved in keto-enol tautomerism)						
13.010 1446	4-Hydroxy-2,5-dimethylfuran-3(2H)-one		0 5203	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.

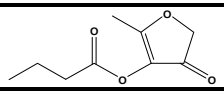
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (µg/*capita*/day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d), (e)]	EFSA conclusion on the named compound: (Procedure steps, intake estimates, NOAEL, genotoxicity)
13.084 1449	2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone		0 13	Class II A3: Intake below threshold	d	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. EFSA allocated the substance to Class III. No safety concern at the estimated level of intake based on the MSDI approach.
13.085 1450	4-Hydroxy-5-methylfuran-3(2H)-one		0 0.07	Class II A3: Intake below threshold	d	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. EFSA allocated the substance to Class III. No safety concern at the estimated level of intake based on the MSDI approach.
13.099 1456	4-Acetoxy-2,5-dimethylfuran-3(2H)-one		400 8	Class II A3: Intake below threshold	d	Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. EFSA allocated the substance to Class III. No safety concern at the estimated level of intake based on the MSDI approach.
13.176 1519	Furaneyl butyrate		4.2 4	Class III No evaluation		Evaluated in FGE.220Rev1, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.

(a): 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.
(b): Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/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 B. QSAR Predictions on Mutagenicity

Table 4: QSAR Predictions on Mutagenicity in Five Models for 10 Ketones from Subgroup 4.4

FL-no JECFA-no	EU Register name	Structural formula [a]	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]
Substances in subgroup 4.4a (Furan-3(2H)-ones)							
13.089 1451	2,5-Dimethyl-4-methoxyfuran-3(2H)-one		OD*	NEG	OD*	OD*	OD*
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2H)-one		OD*	NEG	OD*	OD*	OD*
13.119	2,5-Dimethylfuran-3(2H)-one		OD*	NEG	OD*	OD*	OD*
13.157	5-Methylfuran-3(2H)-one		OD*	NEG	POS	OD*	OD*
13.175	4-Acetyl-2,5-dimethylfuran-3(2H)-one		OD*	NEG	OD*	OD*	OD*
Substances in subgroup 4.4b (Furan-3(2H)-ones in which the α,β-unsaturated double bond can be involved in keto-enol tautomerism)							
13.010 1446	4-Hydroxy-2,5-dimethylfuran-3(2H)-one		OD*	NEG	NEG	OD*	NEG
13.084 1449	2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone		OD*	NEG	NEG	OD*	NEG
13.085 1450	4-Hydroxy-5-methylfuran-3(2H)-one		OD*	NEG	NEG	OD*	NEG
13.099 1456	4-Acetoxy-2,5-dimethylfuran-3(2H)-one		OD*	NEG	OD*	OD*	OD*

FL-no JECFA-no	EU Register name	Structural formula [a]	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]
13.176	Furaneyl butyrate		OD*	NEG	OD*	OD*	NEG

(a): Structure group 4.4: α,β-unsaturated ketones.
(b): Local model on aldehydes and ketones, Ames TA100. (NEG: Negative; POS: Positive; OD*: out of domain).
(c): MultiCase Ames test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(d): MultiCase Mouse Lymphoma test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(e): MultiCase Chromosomal aberration in CHO (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(f): MultiCase Chromosomal aberration in CHL (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
* OD, out of applicability domain: not matching the range of conditions where a reliable prediction can be obtained in this model. These conditions may be physicochemical, structural, biological etc.

Appendix C. Summary of Carcinogenicity and Genotoxicity Studies

Table 5: Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No/Sex/Group	Route	Dose levels	Duration	Results	Reference	Comments
4-hydroxy-2,5-dimethylfuran-3(2H)-one [13.010]	Rats; Male, Female 60/sex/group	Diet	0, 100, 200 or 400 mg/kg bw/day	2 years	Males: No increase in tumour incidences Females: No increases in tumour incidences	(Kelly and Bolte, 2003)	Valid (GLP/OECD compliant) The NOAEL was 200 mg/kg bw/day based on reduced mean body weight at the highest dose.

SUMMARY OF *IN VITRO* GENOTOXICITY DATA CONSIDERED BY THE PANEL IN FGE.220

Table 6: Summary of *in vitro* Genotoxicity Data Considered by the Panel in FGE.220

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments[(e)]
4-hydroxy-2,5-dimethylfuran-3(2H)-one [13.010]	Reversed mutation	*S. typhimurium* TA1535, TA1537, TA1538,TA100 and TA98	10.0, 33.3, 100.0, 333.3, 1000, 2000, 3300, 4000, 6000 and 8000 µg/plate	Positive[(a),(b)]	(Gilroy et al., 1978)	Valid. Unpublished non-GLP study. The report contains sufficient details. Result is considered valid.
	Reversed mutation	*S. typhimurium* TA100 and TA98	0 - 10000 µg/plate	Positive[(a),(b)]	(Hiramoto et al., 1996b)	Valid. Positive in TA 100 (+/– S9); negative in TA 98 (+/- S9).
	Reversed mutation	*S. typhimurium* TA100, TA102, TA98 and TA97	500 - 4000 µg/plate	Positive[(a),(c)]	(Xing et al., 1988)	Limited validity; No methodological details, but stated to be performed according to (Maron and Ames, 1983). Some errors reduce the trustworthiness of the paper.
	Reversed mutation	*E. coli* WP2 uvrA$^-$	10.0, 33.3, 100.0, 333.3, 1000 and 3300 µg/plate	Negative	(Gilroy et al., 1978)	Valid. Unpublished non-GLP study. The report contains sufficient details. Result is considered valid.
	DNA damage	*B. subtilis* H17 (Rec$^+$) and M45 (Rec$^-$)	20, 40, 60, 80 and 120 µg/disc	Positive	(Xing et al., 1988)	Validity cannot be evaluated (Test system with low predictive value for genotoxicity). No methodological details, but stated to be performed according to (Kada et al., 1972).
	DNA strand breaks	pBR322 DNA	2.6 - 780 µmol/l (0.3 - 100 mg/l)	Positive	(Hiramoto et al., 1996b)	Valid. Single strand breaks caused by redox cycling of the substance in combination with metal ions, generating reactive oxygen species.
4-Hydroxy-5-methylfuran-3(2H)-one [13.085]	Reversed mutation	*S. typhimurium* TA100 and TA98	0 - 5000 µg/plate	Positive[(a),(b)]	(Hiramoto et al., 1996a)	Limited validity. Limited due to uncertainty of test substance. Positive in TA 100 (+/– S9); negative in TA 98 (+/- S9).
	DNA strand breaks	pBR322 DNA	0 - 900 µmol/l (0 - 103mg/l)	Positive[(a),(d)]	(Hiramoto et al., 1996a)	Valid. Single strand breaks caused by redox cycling of the substance in combination with metal ions, generating reactive oxygen species.
2,5-Dimethyl-3(2H)-Furanone [13.119]	Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98,TA100 and TA102,	0 - 5000 µg/plate	Negative	(RCC - CCR, 2007)	Valid. According to current guidelines.

2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [13.084]	Reversed mutation	*S. typhimurium* TA100 and TA98	0 – 10000 µg/plate	Positive[(a),(b)]	(Li et al., 1998)	Valid. positive with and without S9 in TA 100; negative in TA98 (+/- S9).
	DNA strand breaks	pBR322 DNA	0 - 2000 µM	Positive[(d)]	(Li et al., 1998)	Valid. Single strand breaks caused by redox cycling of the substance in combination with metal ions, generating reactive oxygen species.

(a): With and without metabolic activation provided by S9 (9000 x g supernatant from rodent liver).
(b): Positive results only observed in TA100.
(c): Positive results in all strains at the highest dose tested.
(d): Only positive without inhibitors of redox cycling and ROS scavengers.
(e): Validity of genotoxicity studies:

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

Table 7: Summary of *in vivo* Genotoxicity Data Considered by the Panel in FGE.220

Chemical Name [FL-no]	Test System	Test Object	Route	Dose mg/kg bw	Reported Result	Reference	Comments[(a)]
4-hydroxy-2,5-dimethylfuran-3(2H)-one [13.010]	Micronucleus formation	Mouse, bone marrow	Not stated	0, 186, 232 or 309	Positive	(Xing et al., 1988)	Limited validity. Important data not given; Reference to methodological description could not be traced.
	Chromosomal aberration	Mouse spermatocytes	Not stated	0, 232, 464 or 928	Positive	(Xing et al., 1988)	Limited validity. Important data not given; Reference to methodological description could not be traced. Predominant aberration: malsegregation of chromosomes.
	Sister chromatid exchange	Mouse, bone marrow	Intra-abdominal injection	0, 185, 232 and 303	Positive	(Xing et al., 1988)	Validity cannot be assessed. Dose-related increase; statistically significant at all dose levels, but max increase < 2-fold. Effect not adequately specified; very intense exposure to BrdU. Non-validated protocol. Relevance for the evaluation of genotoxicity questionable.
	Sister chromatid exchange	Mouse spermatocytes	Oral (gavage)	200, 400 or 800	Positive	(Tian et al., 1992)	Limited validity. Relevance for the evaluation of genotoxicity questionable; non- validated test protocol.
	Micronucleus formation	Mouse early sperm cells	Oral (gavage)	200, 400 or 800	Positive	(Tian et al., 1992)	Limited validity Non-validated test protocol.
	Micronucleus formation	Mouse peripheral blood cells	Gavage	1000, 2000 and 3000	Positive	(Hiramoto et al., 1998)	Valid
	Micronucleus formation	Male mice peripheral erythrocytes	i.p.	500, 1000 and 1500	Positive	(Hiramoto et al., 1996b)	Valid

Table 7: Summary of *in vivo* Genotoxicity Data Considered by the Panel in FGE.220

Chemical Name [FL-no]	Test System	Test Object	Route	Dose mg/kg bw	Reported Result	Reference	Comments[(a)]
2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone [13.084]	Micronucleus formation	Mouse peripheral blood cells	Gavage	0, 1000, 2000 and 3000	positive	(Hiramoto et al., 1998)	Valid
	Micronucleus formation	Male mice peripheral erythrocytes	i.p.	0, 500 and 1000	positive	(Li et al., 1998)	Valid

(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).

SUMMARY OF ADDITIONAL GENOTOXICITY DATA CONSIDERED BY THE PANEL IN FGE.220REV1

Table 8: Summary of Additional Genotoxicity Data Considered by the Panel in FGE.220Rev1

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Reported Result	Reference	Comments[(a)]
4-hydroxy-2,5-dimethylfuran-3(2H)-one [13.010]	Mouse Lymphoma	L5178Ytk+/- mouse lymphoma cells	-	111, 167, 250, 375 and 750 µg/ml	Negative both with and without S9	(Ross and Harris, 1979)	Limited validity. Study not performed according to current guideline. To short treatment and no differentiation between small and large colonies
	Dominant lethal assay in a rat fertility study	Dominant lethals in Crl:CD(SD) male rats (25/group)	Oral gavage	100, 500 and 1000 mg/kg bw/day for 2 weeks(Phase I) and 9 weeks (Phase II)	no increase of dominant lethal effects	(Sloter, 2008)	Valid GLP study in accordance with ICH Guideline 4.1.1.

(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).

A study by Honarvar (Honarvar, 2008) was also submitted. However due to unknown identity of the tested material, this study is not included in the table.

SUMMARY OF ADDITIONAL GENOTOXICITY DATA CONSIDERED BY THE PANEL IN FGE.220REV2 AND REV3

Table 9: Summary of Additional Genotoxicity Data Considered by the Panel in FGE.220Rev2 and Rev3

Chemical Name [FL-no]	Test System	Test Object	Dose	Reported Result	Reference	Comments
2,5-Dimethylfuran-3(2H)-one [13.119]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	3 - 5000 µg/plate [1,2]	Negative	(Sokolowski, 2007)	All strains were negative. Study design complied with current GLP and OECD recommendations. Acceptable top concentration was achieved.
		S. typhimurium TA98, TA100, TA102, TA1535 and TA1537	33 - 5000µg/plate [1,3]	Negative		
	Micronucleus Assay	Human peripheral blood lymphocytes	900 - 1120 µg/mL [1,6] 900 - 1120 µg/mL [4,7]	Negative	(Lloyd, 2011)	The MNBN cell frequencies in all treated cultures fell within the normal range. Complies with draft OECD Guideline 487 and GLP recommendations.
4-Acetyl-2,5-dimethylfuran-3(2H)-one [13.175]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537	0.32 – 5000 µg/plate [1,2]	Negative	(Bowen, 2011)	Evidence of toxicity was observed at 5000µg/plate in all strains in the absence and presence of S-9. Study design complied with current GLP and OECD recommendations.
			78.13 - 5000 µg/plate [2,4] 78.13 - 5000µg/plate [3,5]	Negative		
	Micronucleus Assay	Human peripheral blood lymphocytes	1000 - 1542 µg/mL [1,6] 400 - 900 µg/mL [4,7]	Equivocal	(Lloyd, 2012)	Study in compliance with GLP and OECD recommendations. Statistical significant increase, dose-related, in the presence of S9-mix at all three concentrations in a first experiment. Lower statistical significance at the two highest concentrations in an enlarged scoring, carried out with an unjustified approach. Mean MNBN cell frequencies fell within the historical control range with exception of a single replicate (see main text).

Table 9: Summary of Additional Genotoxicity Data Considered by the Panel in FGE.220Rev2 and Rev3

Chemical Name [FL-no]	Test System	Test Object	Dose	Reported Result	Reference	Comments
			1000, 1250 and 1542 µg/mL [5,6]	Negative	(Lloyd, 2014)	Follow up study in compliance with GLP and OECD recommendations. Statistically significant increase ($p \leq 0.05$) in the mean frequency of micronuclei was reported only at the lowest of the three concentrations tested (1000 µg/mL) but remained well within the normal historical control range values (0.1 to 0.9 %) for both replicate cultures.

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

ABBREVIATIONS

CA	Chromosomal aberration
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
CPA	Cyclophosphamide
DNA	Deoxyribonucleic acid
EFFA	European Flavour Association
EFSA	European Food Safety Authority
EU	European Union
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
MN	Micronuclei
MNBN	MicroNucleated BiNucleate cells
MS	Mass spectra
MSDI	Maximised Survey-derived Daily Intake
MW	Molecular Weight
NMR	Nuclear Magnetic Resonance
No	Number
OECD	Organisation for Economic Co-operation and Development
(Q)SAR	(Quantitative) Structure Activity Relationship
PHA	Phytohaemagglutinin
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food

WHO World Health Organization