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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

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

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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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-pentyldienefuran-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-pentyldienefuran-2(5H)-one [FL-no: 10.042], 2,7-dimethyloctatrans,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 α,β-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.
### Table 1: Specification Summary of the Substances in the present group

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Mol.formula</th>
<th>Mol.weight</th>
<th>Solubility 1)</th>
<th>Solubility in ethanol 2)</th>
<th>Boiling point, °C 3)</th>
<th>Melting point, °C</th>
<th>ID test</th>
<th>Refrac. Index 4)</th>
<th>Spec.gravity 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.023</td>
<td>5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>3153</td>
<td>2300</td>
<td>698-10-2</td>
<td>Liquid</td>
<td>C7H10O3</td>
<td>142.15</td>
<td>Soluble</td>
<td>83-86 (1 hPa)</td>
<td>IR 95 %</td>
<td>1.486-1.493</td>
<td>1.134-1.144</td>
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<tr>
<td>10.030</td>
<td>3-Hydroxy-4,5-dimethylfuran-2(5H)-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>3634</td>
<td>11834</td>
<td>28664-35-9</td>
<td>Liquid</td>
<td>C5H8O3</td>
<td>128.13</td>
<td>Soluble</td>
<td>81 (8 hPa)</td>
<td>25 IR 97.5 %</td>
<td>1.497-1.503</td>
<td>1.058-1.063</td>
<td></td>
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</tr>
<tr>
<td>10.034</td>
<td>5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>3755</td>
<td>80417-97-6</td>
<td>Liquid</td>
<td>C10H12O2</td>
<td>164.20</td>
<td>Slightly soluble</td>
<td>Soluble</td>
<td>264-266 (13hPa)</td>
<td>IR NMR 95 %</td>
<td>1.542-1.548</td>
<td>1.090-1.096</td>
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<td>10.036</td>
<td>5,6,7,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4H)-one</td>
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<td>3764</td>
<td>13341-72-5</td>
<td>Liquid</td>
<td>C10H12O2</td>
<td>164.22</td>
<td>Slightly soluble</td>
<td>Soluble</td>
<td>261-263 (8 hPa)</td>
<td>IR NMR 98 %</td>
<td>1.497-1.503</td>
<td>1.058-1.063</td>
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<td>10.042</td>
<td>3,4-Dimethyl-5-pentylidenefuran-2(5H)-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>4050</td>
<td>11873</td>
<td>774-64-1</td>
<td>Liquid</td>
<td>C11H16O2</td>
<td>180</td>
<td>Soluble</td>
<td>Freely soluble</td>
<td>MS 93 %</td>
<td>1.560-1.575</td>
<td>0.980-1.000</td>
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<tr>
<td>10.043</td>
<td>2,7-Dimethylocta-5(trans),7-dieno-1,4-lactone</td>
<td><img src="image" alt="Structural formula" /></td>
<td>74183-60-1</td>
<td>Liquid</td>
<td>C10H12O2</td>
<td>166.22</td>
<td>Practically insoluble or insoluble</td>
<td>Freely soluble</td>
<td></td>
<td>NMR 95 %</td>
<td>1.453-1.459</td>
<td>0.977-0.983</td>
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<td>10.046</td>
<td>Hex-2-eno-1,4-lactone</td>
<td><img src="image" alt="Structural formula" /></td>
<td>2407-43-4</td>
<td>Liquid</td>
<td>C6H8O2</td>
<td>112.13</td>
<td>Soluble</td>
<td></td>
<td>93 (13 hPa)</td>
<td>MS 95 %</td>
<td>1.431-1.437</td>
<td>1.067-1.073</td>
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<tr>
<td>10.054</td>
<td>Non-2-eno-1,4-lactone</td>
<td><img src="image" alt="Structural formula" /></td>
<td>4188</td>
<td>21963-26-8</td>
<td>Liquid</td>
<td>C5H8O2</td>
<td>154.21</td>
<td>Practically insoluble or insoluble</td>
<td>Freely soluble</td>
<td></td>
<td>196</td>
<td>1.457-1.463</td>
<td>0.981-0.987</td>
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<tr>
<td>10.057</td>
<td>3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3H)-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>4140</td>
<td>57743-63-2</td>
<td>Liquid</td>
<td>C10H14O2</td>
<td>166.22</td>
<td>Practically insoluble or insoluble</td>
<td>Freely soluble</td>
<td></td>
<td>231</td>
<td>13</td>
<td>1.494-1.500</td>
<td>1.053-1.059</td>
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### Table 1: Specification Summary of the Substances in the present group

<table>
<thead>
<tr>
<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Mol.formula</th>
<th>Mol.weight</th>
<th>Solubility 1)</th>
<th>Solubility 2)</th>
<th>Boiling point, °C 3)</th>
<th>Melting point, °C</th>
<th>Refrac. Index 4)</th>
<th>Spec.gravity 5)</th>
</tr>
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<tbody>
<tr>
<td>10.060</td>
<td>2-Decen-1,4-lactone</td>
<td><img src="image" alt="2-Decen-1,4-lactone" /></td>
<td>Liquid</td>
<td>C_{10}H_{16}O_{2}</td>
<td>2518-53-8</td>
<td>Practically insoluble</td>
<td>Freely soluble</td>
<td>145 (13 hPa)</td>
<td>MS 95 %</td>
<td>1.457-1.463</td>
<td>0.976-0.981</td>
<td></td>
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<tr>
<td>10.066</td>
<td>Furan-2(SH)-one</td>
<td><img src="image" alt="Furan-2(SH)-one" /></td>
<td>Liquid</td>
<td>C_{4}H_{4}O_{2}</td>
<td>4138</td>
<td>Soluble</td>
<td>Freely soluble</td>
<td>214</td>
<td>1.457-1.463</td>
<td>1.182-1.188</td>
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<tr>
<td>13.012</td>
<td>6-Methylcoumarin</td>
<td><img src="image" alt="6-Methylcoumarin" /></td>
<td>Solid</td>
<td>C_{10}H_{8}O_{2}</td>
<td>2699 579 92-48-8</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>73-79 IR 99 %</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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 \( \alpha,\beta \)-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 \( \alpha,\beta \)-ketone grouping in conjugation with an aromatic ring, this substance would not be considered a representative for any of the remaining \( \alpha,\beta \)-unsaturated lactones in this subgroup.

In the EFSA opinion “List of \( \alpha,\beta \)-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)

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
</tr>
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<tbody>
<tr>
<td>10.023</td>
<td>5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one</td>
<td></td>
</tr>
<tr>
<td>10.042</td>
<td>3,4-Dimethyl-5-pentylidenefuran-2(5H)-one</td>
<td></td>
</tr>
<tr>
<td>10.066</td>
<td>Furan-2(5H)-one</td>
<td></td>
</tr>
</tbody>
</table>

The Panel viewed the previous JECFA 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).

<table>
<thead>
<tr>
<th>FGE</th>
<th>Adopted by EFSA</th>
<th>Link</th>
<th>No. of Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGE.217Rev1</td>
<td>4 July 2013</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Substance / study type</th>
<th>Ames test</th>
<th>Micronucleus assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-Dimethyl-5-pentylidenefuran-2(5H)-one [FL-no: 10.042]</td>
<td>Bowen, 2011b</td>
<td>Whitwell, 2012a</td>
</tr>
</tbody>
</table>
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 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 ≤ 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 ≤ 0.01), 90, 110 and 120 μg/ml (p ≤ 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 ≤ 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 < 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>
<thead>
<tr>
<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>EU MSDI 1) (µg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>JECFA Outcome on the named compound</th>
<th>EFSA conclusion on the named compound (genotoxicity)</th>
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<tbody>
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<td>10.043</td>
<td>2,7-Dimethylocta-5(trans),7-dieno-1,4-lactone</td>
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<td>0.0012</td>
<td>Class I No evaluation</td>
<td>Not evaluated by JECFA</td>
<td>Evaluated in FGE.217Rev1, additional genotoxicity data required.</td>
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<td>10.054</td>
<td>Non-2-eno-1,4-lactone</td>
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<td>0.61</td>
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<td>Not evaluated by JECFA</td>
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<td>5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one</td>
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<td>Class III A3: Intake below threshold</td>
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</tr>
<tr>
<td>13.012</td>
<td>6-Methylcoumarin</td>
<td></td>
<td>250</td>
<td>Class III B3: Intake above threshold</td>
<td>Data must be available</td>
<td>Adequate data are available to reach the conclusion &quot;No safety concern at the estimated level of intake based on the MSDI approach.&quot;.</td>
<td></td>
</tr>
<tr>
<td>10.042</td>
<td>3,4-Dimethyl-5-pentylidenefuran-2(5H)-one</td>
<td></td>
<td>0.12</td>
<td>Class III No evaluation</td>
<td>Not evaluated by JECFA</td>
<td>Evaluated in FGE.217Rev1, additional genotoxicity data required.</td>
<td></td>
</tr>
<tr>
<td>10.060</td>
<td>2-Decen-1,4-lactone</td>
<td></td>
<td>0.037</td>
<td>Class III No evaluation</td>
<td>Not evaluated by JECFA</td>
<td>Evaluated in FGE.217Rev1, additional genotoxicity data required.</td>
<td></td>
</tr>
<tr>
<td>FL-no</td>
<td>JECFA-no</td>
<td>EU Register name</td>
<td>Structural formula</td>
<td>EU MSDI 1) US MSDI (µg/capita/day)</td>
<td>Class 2) Evaluation procedure path 3)</td>
<td>JECFA Outcome on the named compound [4) or 5)]</td>
<td>EFSA conclusion on the named compound (genotoxicity)</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>------------------</td>
<td>-------------------</td>
<td>----------------------------------</td>
<td>--------------------------------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>10.046</td>
<td>Hex-2-eno-1,4-lactone</td>
<td><img src="image" alt="Formula" /></td>
<td>0.0024</td>
<td>No evaluation</td>
<td>Not evaluated by JECFA</td>
<td>Evaluated in FGE.217Rev1, additional genotoxicity data required.</td>
<td></td>
</tr>
<tr>
<td>10.057</td>
<td>3a,4,5,7a-Tetrahydro-3,6-dimethylbenzofuran-2(3H)-one</td>
<td><img src="image" alt="Formula" /></td>
<td>0.012</td>
<td>No evaluation</td>
<td>Not evaluated by JECFA</td>
<td>Evaluated in FGE.217Rev1, additional genotoxicity data required.</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>FL-no</th>
<th>Subgroup</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no CAS no</th>
<th>ISS Local Model Ames Test TA100</th>
<th>MultiCASE Ames test</th>
<th>MultiCASE Mouse lymphoma test</th>
<th>MultiCASE Chromosoma l aberration test in CHO</th>
<th>MultiCASE Chromosoma l aberration test in CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.054</td>
<td>4.1</td>
<td>Non-2-eno-1,4-lactone</td>
<td><img src="image" alt="Structure" /></td>
<td>4188-21963-26-8</td>
<td>OD NEG OD EQU OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.060</td>
<td>4.1</td>
<td>2-Decen-1,4-lactone</td>
<td><img src="image" alt="Structure" /></td>
<td>2518-53-8</td>
<td>OD NEG OD EQU OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.066</td>
<td>4.1</td>
<td>Furan-2(5H)-one</td>
<td><img src="image" alt="Structure" /></td>
<td>4138-</td>
<td>OD NEG POS EQU EQU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.034</td>
<td>4.1</td>
<td>5,6-Dihydro-3,6-dimethylbenzofuran-2(4H)-one</td>
<td><img src="image" alt="Structure" /></td>
<td>3755-80417-97-6</td>
<td>OD NEG OD OD OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.036</td>
<td>4.1</td>
<td>5,6,7a-Tetrahydro-3,6-dimethylbenzofuran-2(4H)-one</td>
<td><img src="image" alt="Structure" /></td>
<td>3764-13341-72-5</td>
<td>OD NEG OD OD OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.012</td>
<td>4.1</td>
<td>6-Methylcoumarin</td>
<td><img src="image" alt="Structure" /></td>
<td>2699-579-92-48-8</td>
<td>OD POS OD OD OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.023</td>
<td>4.1</td>
<td>5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one</td>
<td><img src="image" alt="Structure" /></td>
<td>3153-2300-698-10-2</td>
<td>OD NEG NEG NEG NEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.030</td>
<td>4.1</td>
<td>3-Hydroxy-4,5-dimethylfuran-2(5H)-one</td>
<td><img src="image" alt="Structure" /></td>
<td>3634-11834-28664-35-9</td>
<td>OD NEG NEG NEG NEG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.042</td>
<td>4.1</td>
<td>3,4-Dimethyl-5-pentyldenefuran-2(5H)-one</td>
<td><img src="image" alt="Structure" /></td>
<td>4050-11873-774-64-1</td>
<td>OD OD OD OD OD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4: QSAR Predictions on Mutagenicity in Five Models for 10 Lactones from Subgroup 4.1 and two precursors

<table>
<thead>
<tr>
<th>FL-no</th>
<th>Subgroup</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>ISS Local Model Ames Test TA100</th>
<th>MultiCASE Ames test</th>
<th>MultiCASE Mouse lymphoma test</th>
<th>MultiCASE Chromosomal aberration test in CHO</th>
<th>MultiCASE Chromosomal aberration test in CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.046</td>
<td>4.1</td>
<td>Hex-2-eno-1,4-lactone</td>
<td><img src="image" alt="Structural formula" /></td>
<td>-</td>
<td>OD</td>
<td>2407-43-4</td>
<td>NEG</td>
<td>OD</td>
<td>POS</td>
<td>OD</td>
<td>OD</td>
</tr>
<tr>
<td>Not in Register</td>
<td>2.6</td>
<td>3-methyl-6-(1-carboxyethyl)-2-cyclohexen-1-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>-</td>
<td>OD</td>
<td>-</td>
<td>NEG</td>
<td>OD</td>
<td>OD</td>
<td>NEG</td>
<td>EQU</td>
</tr>
<tr>
<td>Not in Register</td>
<td>1.2.4</td>
<td>2,7-dimethyl-4-oxo-oct-5,7-dienoic acid</td>
<td><img src="image" alt="Structural formula" /></td>
<td>NYA</td>
<td>NYA</td>
<td>NYA</td>
<td>NYA</td>
<td>NYA</td>
<td>NYA</td>
<td>NYA</td>
<td>NYA</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Species; Sex No./Group</th>
<th>Route</th>
<th>Dose levels</th>
<th>Duration</th>
<th>Results</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Methylcoumarin [13.012]</td>
<td>Rat; Male, Female 25/sex/group</td>
<td>Diet</td>
<td>0, 25, 50, 175, 250, 375 or 750 mg/kg bw/day</td>
<td>2 years</td>
<td>Males and females: No increases in tumour incidences</td>
<td>(Hagan et al., 1967)</td>
<td>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.</td>
</tr>
</tbody>
</table>
### GENOTOXICITY (*IN VITRO*)

**Table 6: Genotoxicity (*in vitro*)**

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

<sup>a</sup>: With and without metabolic activation.<br>
<sup>b</sup>: Pre-incubation method.<br>
<sup>c</sup>: With metabolic activation.<br>
<sup>d</sup>: Validity of genotoxicity studies:<br>
  - Valid.<br>
  - Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and/or limited documentation).<br>
  - 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).<br>
  - Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).
**GENOTOXICITY (IN VIVO)**

**Table 7:** Genotoxicity *(in vivo)*

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

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

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Test System in vitro</th>
<th>Test Object</th>
<th>Concentrations of Substance and Test Conditions</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ethyl-3-hydroxy-4-</td>
<td>Reverse Mutation</td>
<td>S. typhimurium TA98, TA100, TA1535</td>
<td>1.6, 8, 40, 200, 1000 and 5000 μg/plate [1,2]</td>
<td>Negative</td>
<td>(Bowen, 2011a)</td>
<td>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.</td>
</tr>
<tr>
<td>methylfuran-2(5H)-one [10.023]</td>
<td></td>
<td>and TA102</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4-Dimethyl-5-</td>
<td>Reverse Mutation</td>
<td>S. typhimurium TA98, TA100, TA1535</td>
<td>0.32, 1.6, 8, 40, 200, 1000 and 5000 μg/plate [1,2]</td>
<td>Negative</td>
<td>(Bowen, 2011b)</td>
<td>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. 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.</td>
</tr>
<tr>
<td>pentylidenefuran-2(5H)-one</td>
<td></td>
<td>and TA1537</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[10.042]</td>
<td></td>
<td>and TA102</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4-Dimethyl-5-</td>
<td>Reverse Mutation</td>
<td>S. typhimurium TA98, TA100, TA1535</td>
<td>78.13 - 5000 μg/plate [2,3]</td>
<td>Negative</td>
<td></td>
<td>Evidence of toxicity was observed in all strains in the absence and presence of S9 at 200 μg/plate and above. 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.</td>
</tr>
<tr>
<td>pentylidenefuran-2(5H)-one</td>
<td></td>
<td>and TA1537</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[10.042]</td>
<td></td>
<td>and TA102</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NEW GENOTOXICITY (IN VITRO)**
### Table 8: Summary of Additionally Genotoxicity Data [FL-no: 10.023, 10.042 and 10.066] of subgroup 4.1

<table>
<thead>
<tr>
<th>Chemical Name [FL-no:]</th>
<th>Test System in vitro</th>
<th>Test Object</th>
<th>Concentrations of Substance and Test Conditions</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro Micronucleus induction</strong></td>
<td>Human peripheral blood lymphocytes</td>
<td>40, 60, 70 and 90 μg/ml [3,6]</td>
<td>Equivocal</td>
<td>(Whitwell, 2012a) and in compliance with GLP.</td>
<td>Valid study in accordance with OECD Guideline 487 (OECD, 2010) and in compliance with GLP.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60, 90, 110, and 140 μg/ml [5,6]</td>
<td>Positive</td>
<td>Whitwell (2012a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10, 13, and 15 μg/ml [3,7]</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furan-2(5H)one [10.066]</td>
<td><strong>Reverse Mutation</strong></td>
<td>S. typhimurium TA98, TA100, TA1535, TA1537 and TA102</td>
<td>0.32, 1.6, 8, 40, 200, 1000, and 5000 μg/plate [1,2]</td>
<td>Negative</td>
<td>(Bowen, 2011c)</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>156.3, 312.5, 625, 1250, 2500 and 5000 μg/plate [2,3]</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>156.3, 312.5, 625, 1250, 2500 and 5000 μg/plate [4,5]</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In vitro Micronucleus induction</strong></td>
<td>Human peripheral blood lymphocytes</td>
<td>200, 350, 425, 450 and 475 μg/ml [3,6]</td>
<td>Equivocal</td>
<td>(Whitwell, 2012b)</td>
<td>Valid study in accordance with OECD Guideline 487 (OECD, 2010) and in compliance with GLP.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100, 250, 425, 450 and 475 μg/ml [5,6]</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10, 50, 60, 67.5 and 72.5 μg/ml [3,7]</td>
<td>Equivocal</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[1] With and without S9 metabolic activation.
REFERENCES


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