Scientific opinion of Flavouring Group Evaluation 205 Revision 1 (FGE.205Rev1): consideration of genotoxicity data on representatives for 13 α,β-unsaturated aliphatic ketones with terminal double bonds and precursors from chemical subgroup 1.2.2 of FGE.19

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

Abstract

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was requested to consider in the Flavouring Group Evaluation 205 (FGE.205), the additional data on genotoxicity submitted by the Industry on two representative substances, oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], from subgroup 1.2.2 of FGE.19. The Panel concluded that both substances were weakly genotoxic in bacteria with pent-1-en-3-one being the most potent (previously available data). In these assays, the representative substances were highly cytotoxic with a steep toxicity curve and with a very narrow concentration range resulting in mutagenicity. Both substances were also tested in mammalian cells for gene mutations at the hprt locus and for structural and numerical chromosomal aberrations in the micronucleus assay. Also in mammalian cells, the test substances were highly cytotoxic. The Panel considered that the positive effects in the bacterial mutagenicity assays of the two representative substances cannot be overruled by the one negative and one equivocal gene mutation test in mammalian cells. Therefore, the Panel recommended to test the most potent of the representative substances, pent-1-en-3-one, in an in vivo Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver. The Industry has now submitted new data, a combined micronucleus and Comet assay for pent-1-en-3-one, with scoring in the liver and duodenum, and an Ames test and a Comet assay with scoring in the liver for oct-1-en-3-one. Based on these new data, the Panel concluded that the concern for a genotoxic potential could be ruled out for the two representative substances and accordingly for the remaining 11 substances in FGE.205Rev1. The 13 substances in FGE.205Rev1 can therefore be evaluated via the Procedure.

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Keywords: α,β-unsaturated aliphatic ketones, terminal double bond, flavouring substances, safety evaluation, FGE.205, subgroup 1.2.2, FGE.19

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Summary

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000.

Flavouring Group Evaluation 205 (FGE.205), corresponding to subgroup 1.2.2 of FGE.19, concerns four $\alpha,\beta$-unsaturated aliphatic ketones with a terminal double bond and nine precursors for such ketones. The 13 substances under consideration in the present evaluation are $\alpha,\beta$-unsaturated ketone structures (or can be metabolised to such) which are considered to be structural alerts for genotoxicity. The data on genotoxicity previously available did not rule out the concern for genotoxicity. The Panel has identified two substances in subgroup, 1.2.2, oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], which will represent the other 11 substances in this subgroup. For these two substances, genotoxicity data according to the test strategy worked out by the Panel have been requested.

The Industry has subsequently submitted data concerning genotoxicity studies for the two representative substances of subgroup 1.2.2.

According to these data, both oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102] were mutagenic in bacteria, and highly cytotoxic with a steep toxicity curve and with a very narrow concentration range resulting in mutagenicity. Both substances were also tested in mammalian cells for gene mutations at the hypoxanthine-guanine phosphoribosyl transferase (hprt) locus and for structural and numerical chromosomal aberrations in the micronucleus assay. Also in mammalian cells, the test substances were highly cytotoxic. In the first opinion on FGE.205, the Panel considered that the positive effects in the bacterial mutagenicity assays of the two representative substances cannot be overruled by the one negative and one equivocal gene mutation test in mammalian cells. Therefore, the Panel recommended to test the most potent of the representative substances, pent-1-en-3-one, in an in vivo Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver.

In response to the request in FGE.205, the Industry has now submitted new data, a combined micronucleus and Comet assay for pent-1-en-3-one with scoring in the liver and duodenum, and an Ames test and a Comet assay with scoring in the liver for oct-1-en-3-one. Based on these new data, the Panel concluded that the concern for a genotoxic potential could be ruled out for the two representative substances [FL-no: 07.081 and 07.102] and accordingly for the remaining 11 substances [FL-no: 02.023, 02.099, 02.104, 02.131, 02.136, 02.155, 02.187, 07.161, 07.210, 09.281 and 09.282] in FGE.205Rev1. These substances can now be evaluated via the Procedure.
# Table of contents

Abstract .................................................................................................................................................. 1
Summary ................................................................................................................................................ 3
1. Introduction ................................................................................................................................5
   1.1. Background and Terms of Reference as provided by the European Commission ................. 5
      1.1.1 Terms of Reference ............................................................................................................. 5
2. Data and methodologies .............................................................................................................. 5
   2.1. History of the evaluation of FGE.19 substances .................................................................. 5
   2.2. Presentation of the substances in flavouring group evaluation 205 ...................................... 6
   2.3. History of the evaluation of the substances belonging to FGE.205 ................................... 7
   2.4. Additionally genotoxicity data considered by the Panel in FGE.205 ................................... 7
      2.4.1. *In vitro* data ..................................................................................................................... 7
      2.4.1.1. Oct-1-en-3-one (amyl vinyl ketone) [FL-no: 07.081] .................................................. 7
      2.4.1.2. Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102] - previously available *in vitro* data 10
      2.4.1.3. Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102] - new available in vitro data ........ 10
      2.4.2. *In vivo* data .................................................................................................................... 11
      2.4.3. Conclusion by the CEF Panel in FGE.205 ........................................................................ 12
3. Assessment ................................................................................................................................. 12
   3.1. Additionally genotoxicity data considered by the Panel in FGE.205 Rev1 ............................. 12
      3.1.1. Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102] .................................................. 12
      3.1.1.1. Micronucleus arm of the assay ....................................................................................... 13
      3.1.1.2. Comet arm of the assay ............................................................................................... 13
      3.1.1.3. Conclusion for pent-1-en-3-one ..................................................................................... 13
      3.1.2. Oct-1-en-3-one (amyl vinyl ketone) [FL-no: 07.081] .................................................. 14
      3.1.2.1. Bacterial reverse mutation assay .................................................................................. 14
      3.1.2.2. Comet assay ............................................................................................................... 14
      3.1.2.3. Conclusion for oct-1-en-3-one ..................................................................................... 15
4. Overall conclusion ....................................................................................................................... 15
   Summary of specifications ............................................................................................................ 16
   Summary of safety evaluation of the JECFA substances in FGE.205 ............................................ 17
   Genotoxicity data considered by the Panel in FGE.205 ............................................................... 18
   Documentation provided to EFSA ................................................................................................. 21
   References .................................................................................................................................... 22
   Abbreviations ............................................................................................................................. 22
1. Introduction

1.1. Background and Terms of Reference 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.

On 27 September 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 205 (FGE.205): consideration of genotoxic potential on α,β-unsaturated aliphatic ketones with terminal double bonds and precursors from chemical subgroup 1.2.2 of FGE.19.

The Panel concluded that for the two representative substances oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], the positive effects in the bacterial mutagenicity assays cannot be overruled by one negative and one equivocal gene mutation test in mammalian cells. Accordingly, an in vivo Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver is requested for the most potent substance, pent-1-en-3-one. As an alternative, a transgenic animal assay would also be acceptable.

On 10 March 2015, the applicant submitted additional studies on the representative substances [FL-no: 07.102] and [FL-no: 07.081]. These studies are intended to cover the substances in this group, namely: FL-nos: 02.023, 02.099, 02.104, 02.131, 02.136, 02.155, 02.187, 07.161, 07.210, 09.281 and 09.282.

1.1.1. Terms of Reference

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 the above mentioned flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

2. Data and methodologies

2.1. History of the evaluation of FGE.19 substances

Flavouring Group Evaluation 19 (FGE.19) contains 360 flavouring substances from the EU Register 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 (QSAR) 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 using two ISS Local Models (Benigni and Netzeva, 2007a,b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, in vitro and in vivo, as well as data on carcinogenicity for several substances. Based on these data, the 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, 2008b) could not be evaluated through the Procedure due to concern with respect to genotoxicity. Corresponding to these subgroups, 15 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 were evaluated using the Procedure. For all or some of the substances in the remaining FGEs, FGE.201, 203, 210, 212, 213, 216, 217 and 220, the genotoxic potential could not be ruled out.

To ease the data retrieval of the large number of structurally related \(\alpha,\beta\)-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, a 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 FGE concerns the evaluation of these data requested on genotoxicity.

### 2.2. Presentation of the substances in flavouring group evaluation 205

Flavouring Group Evaluation 205 (FGE.205), corresponding to subgroup 1.2.2 of FGE.19, concerns four \(\alpha,\beta\)-unsaturated aliphatic ketones with a terminal double bond and nine precursors for such ketones. The 13 substances under consideration in the present evaluation are listed in Table 3.

Nine of the 13 substances have previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at their 59th and 69th meetings (JECFA, 2002, 2009). A summary of their current evaluation status by JECFA and the outcome of this consideration is presented in Table 4.

The \(\alpha,\beta\)-unsaturated aldehyde and ketone structures are considered to be structural alerts for genotoxicity (EFSA, 2008a) and the data on genotoxicity previously available did not rule out the concern for genotoxicity.

The Panel has identified two substances in subgroup 1.2.2 which will represent the other 11 substances in this subgroup (EFSA, 2008c). For these two substances, genotoxicity data according to the test strategy (EFSA, 2008b) have been requested. The representative substances are listed in Table 1.

<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>
</tr>
</thead>
<tbody>
<tr>
<td>07.081</td>
<td>1148</td>
<td>Oct-1-en-3-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>3515</td>
<td>2312</td>
<td>4312-99-6</td>
</tr>
<tr>
<td>07.102</td>
<td>1147</td>
<td>Pent-1-en-3-one</td>
<td><img src="image" alt="Structural formula" /></td>
<td>3382</td>
<td>11179</td>
<td>1629-58-9</td>
</tr>
</tbody>
</table>

2.3. History of the evaluation of the substances belonging to FGE.205

Subgroup 1.2.2 was one of the FGE.19 subgroups for which the Panel concluded that, based on the available data, additional genotoxicity data were necessary to perform the risk assessment for these substances (EFSA, 2008a).

In 2012, the Industry submitted genotoxicity data for the two representative substances. Oct-1-en-3-one [FL-no: 07.081] was tested in an Ames test, a hypoxanthine-guanine phosphoribosyl transferase (hprt) assay and an in vitro micronucleus assay. Pent-1-en-3-one [FL-no: 07.102] was tested in an in vitro micronucleus assay and a hprt assay. These studies were evaluated in FGE.205 (EFSA CEF Panel, 2012) where the Panel concluded that the positive effects in the bacterial mutagenicity assays of the two representative substances cannot be overruled by the one negative and one equivocal gene mutation test in mammalian cells. The Panel recommended to test in vivo the most potent of the representative substances, pent-1-en-3-one, in a Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver.

<table>
<thead>
<tr>
<th>FGE</th>
<th>Adopted by EFSA</th>
<th>Link</th>
<th>No. of substances</th>
</tr>
</thead>
</table>

FGE: Flavouring Group Evaluation; EFSA: European Food Safety Authority.

The applicant has submitted two in vivo studies: a combined micronucleus assay and comet assay for pent-1-en-3-one [FL-no: 07.102] and a comet assay for oct-1-en-3-one [FL-no: 07.081].

The present Revision 1 of FGE.205 (FGE.205Rev1) concerns the evaluation of a combined bone marrow micronucleus test and Comet assay with scoring in the liver and duodenum of rats for pent-1-en-3-one, and an Ames test and a Comet assay with scoring in the liver of rats for oct-1-en-3-one.

Section 2.4 is identical to the text presented in FGE.205. Text presented in Section 3 is the evaluation of the new data in FGE.205Rev1.

2.4. Additionally genotoxicity data considered by the Panel in FGE.205

The Industry has submitted data concerning genotoxicity studies (EFFA, 2011) for the two representative substances of this subgroup:

- Oct-1-en-3-one (amyl vinyl ketone) [FL-no: 07.081]
- Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102]

2.4.1. In vitro data

An Ames test, a hprt assay and an in vitro micronucleus assay have been performed with oct-1-en-3-one [FL-no: 07.081]. An in vitro micronucleus assay and a hprt assay have been performed with pent-1-en-3-one [FL-no: 07.102]. Besides these new studies submitted by Industry, some older studies already considered by the Panel (EFSA, 2008a) with pent-1-en-3-one were included in the submission. An overview of the studies are summarised in Table 5.

2.4.1.1. Oct-1-en-3-one (amyl vinyl ketone) [FL-no: 07.081]

Bacterial reverse mutation assay

An Ames assay was conducted with oct-1-en-3-one in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 to assess the mutagenicity of oct-1-en-3-one, both in the absence and in the presence of metabolic activation by S9-mix, in three experiments. An initial toxicity range-finding experiment was carried out in the absence and in the presence of S9-mix in strain TA100 only, using final concentrations of oct-1-en-3-one at 1.6, 8, 40, 200, 1,000 and 5,000 µg/plate, plus negative (solvent) and positive controls. Evidence of toxicity was apparent on all plates treated at 200 µg/plate and above in the absence and in the presence of S9-mix. Based on this toxicity data, the following concentrations were used for all tester strains in the first experiment: 0.32, 1.6, 8, 40, 200 and 1,000 µg/plate. Following these treatments, evidence of toxicity was observed in all strains at concentrations of 200 and/or 1,000 µg/plate, both in the absence and in the presence of S9-mix.

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4 Data presented in Section 2.4 are cited from the first opinion on FGE.205.
Negative results were obtained for all strains with and without S9-mix, except for TA100 with S9-mix where a statistically significant increase in the number of revertants above the control was observed at 40 μg/plate where the number of revertants increased 1.2-fold and at 200 μg/plate with an increase of 4.6-fold. Toxicity was observed at 1,000 μg/plate (Beevers, 2009).

In the second experiment, plate incorporation treatments of all the tester strains were performed in the absence of S9-mix, with the maximum test concentration reduced to 500 μg/plate to account for a revised estimate of the toxicity limit. In addition, all treatments in the presence of S9-mix were further modified by the inclusion of a preincubation step to increase the range of mutagenic detection. Following these treatments, evidence of toxicity was observed in all strains at concentrations of 125 or 250 μg/plate and above in the absence of S9-mix, and at 62.5 μg/plate and above in the presence of S9-mix. No statistically significant increases in the number of revertants were seen in any strain except TA100. Without S9-mix, the number of revertants in strain TA100 increased 1.2- and 1.4-fold at 62.5 and 125 μg/plate, respectively, and with S9-mix treatment a 1.2-fold increase was observed at 31.25 μg/plate. These increases are below the threshold that is normally considered biologically relevant, which is a twofold increase threshold. In addition to the preincubation treatments described above (with S9-mix), plate incorporation treatments of strain TA100 in the presence of S9-mix were performed at 50, 100, 200, 300, 400 and 500 μg/plate. Evidence of toxicity was observed on all plates treated at 300 μg/plate and above, but there were no statistically significant increases in revertant numbers at any concentration.

In the third experiment, only the TA100 strain was evaluated as it was weakly positive in the previous experiments. Plate incorporation treatments in the absence and presence of S9-mix were performed at 100, 125, 150, 175, 200, 225, 250 and 300 μg/plate. Preincubation treatments solely in the presence of S9-mix were performed at 15, 30, 45, 60, 75, 90 105 and 120 μg/plate. Following these treatments, evidence of toxicity was observed at 225 or 250 μg/plate and above (plate incorporation treatments in the absence or presence of S9-mix, respectively) and at 60 μg/plate (preincubation treatments). The plate incorporation method resulted in increased revertants by 2.3- to 3-fold at treatment concentrations of 125–200 μg/plate in the absence of S9-mix, and 2.2- to 2.9-fold at treatment concentrations of 100–200 μg/plate in the presence of S9-mix. In both cases, the increases were not concentration related. Using preincubation methodology, a 1.7-fold increase in revertants was observed at 45 μg/plate treatments in the presence of S9-mix.

Overall, small but statistically significant (Dunnett’s test, 1% level) increases in revertant numbers were observed following oct-1-en-3-one treatments of strain TA100 both in the absence and in the presence of metabolic activation by S9-mix. This weak mutagenic response was not reproduced on every experimental occasion, but where significant increases were observed they were small in magnitude and limited by toxicity at the next highest oct-1-en-3-one concentrations. The lack of consistent reproducibility of this weak mutagenic response was attributed to variation in toxicity among experiments, and to a small window where mutagenic responses could be observed.

The Panel concluded that oct-1-en-3-one is a weak inducer of mutations in the TA100 strain of S. typhimurium when tested up to toxic concentrations in the absence and in the presence of a rat liver metabolic activation system.

hprt assay

In the light of the weak positive result in the Ames test, it was deemed relevant by the applicant to assess oct-1-en-3-one for its ability to induce mutation at the hprt locus in mouse lymphoma cells. The study consisted of two cytotoxicity range-finding experiments followed by three separate experiments. Two experiments were conducted for 3 h in the absence and in the presence of metabolic activation by S9-mix. This weak mutagenic response was not reproduced on every experimental occasion, but where significant increases were observed they were small in magnitude and limited by toxicity at the next highest oct-1-en-3-one concentrations. The lack of consistent reproducibility of this weak mutagenic response was attributed to variation in toxicity among experiments, and to a small window where mutagenic responses could be observed.
(MF; 4.23 mutants per $10^6$ viable cells compared to the mean vehicle control MF of 1.10 mutants per $10^6$ viable cells) was observed at the highest concentration (2 $\mu$g/mL), but there was no statistically significant linear trend (Lloyd, 2011c).

In the second experiment using 3-h treatment, 10 concentrations ranging from 0.5 to 3 $\mu$g/mL in the absence of S9-mix, and from 0.5 to 4.5 $\mu$g/mL in the presence of S9-mix, were tested. As the highest concentration evaluated in the first experiment (2 $\mu$g/mL) resulted in a RS < 10%, the highest concentration evaluated in the second experiment was 1.8 $\mu$g/mL in the absence of S9-mix (11% RS) and 4.5 $\mu$g/mL in the presence of S9-mix (24% RS). There were no statistically significant increases in MF at any concentration analysed or linear trends. The small increase in MF observed in the first experiment was therefore not reproduced (Lloyd, 2011c).

In the third experiment using 24-h treatment in the absence of S9-mix, 11 concentrations ranging from 0.1 to 2.5 $\mu$g/mL were tested. Seven days after treatment, the highest concentration not too toxic for selection (1.2 $\mu$g/mL) resulted in 16% RS. Mutant frequencies in all treated cultures were lower than in the concurrent control (Lloyd, 2011c).

In this study, high cytotoxicity was observed, which complicates the evaluation of oct-1-en-3-one for genotoxicity in mammalian cells. Some indication of genotoxicity was observed in the absence of S9-mix at the highest non-toxic concentration tested (2 $\mu$g/mL) and 3-h treatment, which however could not be reproduced in the second experiment using the same treatment conditions and a third experiment using 24-h treatment. It is therefore concluded that this gene mutation test is negative under the experimental conditions performed.

In vitro micronucleus assays

Oct-1-en-3-one was tested in an in vitro micronucleus assay using duplicate human lymphocyte cultures prepared from the pooled blood of two female donors both in the absence and in the presence of metabolic activation (S9-mix).

After stimulation for 48 h with phytohaemagglutinin (PHA), cells were treated with oct-1-en-3-one for 3 h (followed by 21 h recovery) with and without S9-mix and for 24 h without S9-mix. An initial range-finding experiment using 4.6–1,262 $\mu$g/mL oct-1-en-3-one (i.e. up to 10 mM) was performed. Based on the cytotoxicity data obtained in this study, measured by effect on the replication index (RI), concentrations ranging from 2 to 20 $\mu$g/mL (absence of S9-mix) or 5 to 40 $\mu$g/mL (presence of S9-mix) were chosen for the main experiment with 3-h treatment. The 3 + 21 h treatment in the absence of S9-mix resulted in 81–99% toxicity at 10 $\mu$g/mL and above. Concentrations of 2, 4, and 8 $\mu$g/mL resulted in 0.20–0.35% micronucleated binucleated (MNBN) cell frequency which was below that of the concurrent control. The 3 + 21 h treatment in the presence of S9-mix resulted in 81% cytotoxicity or greater at concentrations of 20 $\mu$g/mL and above. Treatments at 5, 10 and 15 $\mu$g/mL resulted in MNBN cell frequencies (0.3–0.55%) similar to that of the control (0.5%) (Lloyd, 2011b).

Since the 3-h treatment produced negative results for micronucleus (MN) induction, treatments were also performed over a 24-h period in the absence of S9. In the range-finding experiment, concentrations again ranged up to 1,262 $\mu$g/mL (10 mM) but cells did not replicate (zero RI) at concentrations of 35.33 $\mu$g/mL and above. Also, there was 94% toxicity at 12 $\mu$g/mL. Therefore, 10 $\mu$g/mL was chosen as the top concentration, where RI was reduced by 62%. Treatment at this concentration resulted in a statistically significant ($p < 0.01$) increase in mean MNBN cell frequency to 1.65% (concurrent control was 0.65%). However, the MNBN frequency only exceeded the normal range in one of the two replicate cultures, and there was no increase in MNBN frequency at 8 $\mu$g/mL where reduction in RI was 58%, or any of the lower concentrations. Given that the only positive response was in one replicate at toxicity exceeding 60% and that at the recommended range toxicity (58%), there was no increase in MN frequency, this is considered to be an indirect consequence of high levels of toxicity and is not considered a biologically relevant positive response.

Taken as a whole, treatment with oct-1-en-3-one at acceptable levels of toxicity resulted in frequencies of MNBN cells that were generally similar to (and not significantly different from) those observed in concurrent vehicle controls. It was concluded that oct-1-en-3-one did not induce micronuclei in cultured human peripheral blood lymphocytes when tested up to toxic concentrations for 3 + 21 h in the absence and in the presence of S9-mix and for 24 + 0 h in the absence of S9-mix (Lloyd, 2011b).
2.4.1.2. Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102] – previously available in vitro data

**Bacterial reverse mutation assay**

In a study by Deininger et al. (1990), pent-1-en-3-one was tested for the induction of gene mutations in *S. typhimurium* strain TA100 both with and without Aroclor-induced rat liver S9-mix. The preincubation method was used. The substance, dissolved in DMSO, was tested up to 2 μmol/plate. In the absence of S9-mix, toxicity was observed at a concentration of 0.6 μmol/plate while no toxicity was observed with S9-mix up to the highest tested concentration. Ethyl vinyl ketone was clearly genotoxic both with and without S9-mix. The specific mutagenicity, calculated as the linear slope of the dose–response curve, was 1,293 revertants per μmol without S9-mix and 748 revertants per μmol with S9-mix. Although the peak revertant rate was higher with S9-mix (1,250 revertants at about 2 μmol/plate) than without S9-mix (675 revertants at 0.5 μmol/plate), the specific mutagenicity was higher in the absence of S9-mix. This indicates that the effect of S9-mix is detoxification. However, in supplementary studies with the enzyme inhibitor SKF 525 (an inhibitor of monoxygenase), the mutagenic response disappeared completely, whereas an addition of trichloropropene oxide (TCPO, an inhibitor of epoxide hydrolase) resulted in an increase in mutagenic activity, indicating that epoxidation of the double bond by S9-mix could also play a role in the mutagenicity of pent-1-en-3-one. This data was also included in a paper from the same research group together with data for other α,β-unsaturated ketones and aldehydes (Eder et al., 1993). The same study group also isolated and characterised guanine and deoxyguanosine adducts with pent-1-en-3-one, indicating that pent-1-en-3-one can form DNA adducts, which can give rise to mutations (Eder et al., 1991, 1993). The mutagenic effect in *S. typhimurium* TA100 was supported by genotoxicity in the SOS chromotest performed with the *Escherichia coli* strain PQ37 both with and without S9-mix. Genotoxicity was only observed in the presence of S9-mix. The maximum induction factor was 1.83 which is above the limit of 1.5 for a positive response (Deininger et al., 1990; Eder et al., 1991, 1993). Pent-1-en-3-one was more genotoxic in the SOS chromotest than methyl vinyl ketone (Eder et al., 1993).

Based on these studies, it was concluded that pent-1-en-3-one is genotoxic in bacteria.

2.4.1.3. Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102] – new available in vitro data

Because of the previously reported positive Ames test with pent-1-en-3-one (Deininger et al., 1990), a mammalian cell gene mutation test was performed in mouse lymphoma L5178Y cells and evaluated for induction of forward mutations at the *hprt* locus.

**hprt assay**

Induction of forward mutations at the *hprt* locus after treatment with pent-1-en-3-one in the absence and in the presence of S9-mix was evaluated. Concentrations for the main experiment were established by a preliminary range-finding cytotoxicity experiment. In the first mutation experiment, cell cultures treated with pent-1-en-3-one for 3 h at 0.6, 0.7, 0.8, 0.85, 0.95 and 1.0 μg/mL in the absence of S9-mix and at 1.5, 2, 2.5, 3, 3.25, 3.5, 3.75, 4, 4.5 and 5 μg/mL in the presence of S9-mix were evaluated. Per cent relative survival (%RS) decreased to 16% and 19% at the highest concentrations of 0.95 and 1.0 μg/mL treatment without S9-mix, respectively. No significant increases in MF were observed at any concentration in the absence of S9-mix. In the presence of S9-mix, the highest concentration, 5 μg/mL, resulted in 21% RS. One statistically significant (Dunnett’s test, 5% level) increase in MF from the control (2.48 mutants per 10⁶ viable cells) was observed at the second highest concentration, 4.5 μg/mL (8.7 mutants per 10⁶ viable cells) in the presence of S9-mix in both cultures. A statistically significant linear trend was also observed, although the MF value at the highest concentration tested (5 μg/mL) was not significantly different from the solvent control (5.42 mutants per 10⁶ viable cells) (Lloyd, 2011a).

Thus, additional experiments were undertaken evaluating MF after 3-h treatment with pent-1-en-3-one in the presence of S9-mix but also after 24-h treatment in the absence of S9-mix (two separate experiments). In the presence of S9-mix, the highest concentration evaluated (7 μg/mL) reduced RS to 8%, and therefore exceeded the required level of toxicity. There were no statistically significant increases in MF at any concentration analysed and no statistically significant linear trend. Cultures treated at 4.5 μg/mL gave a 41% RS compared to a 36% RS in first experiment and did not result in increased mutation frequency. The significant increase in mutation frequency observed at a single concentration in the first experiment was not reproduced in the second experiment under the same conditions.
treatment conditions even at higher and more toxic concentrations (the maximum concentrations analysed were 5 and 7 μg/mL in the first and second experiments, respectively). Following 24-h treatment in the absence of S9-mix, the top concentrations evaluated (1.0 and 0.8 μg/mL in the two separate experiments) reduced RS to 12% in each case, and therefore achieved the required level of toxicity for a robust test. There were no statistically significant increases in MF at any concentration and no significant linear trend in either of the two 24-h experiments. The Panel noted that in this in vitro assay pent-1-en-3-one is also cytotoxic to mouse lymphoma L5178Y cells, which could mask a genotoxic effect. Also, in this hprt assay, there was some indication of genotoxicity at the second highest concentration and a linear trend in the presence of S9-mix after 3-h treatment. Although this positive effect was not reproduced in a second experiment using the same experimental design and a third experiment using 24-h treatment without S9-mix, it was concluded by the Panel that the results in this in vitro gene mutation test is equivocal (Lloyd, 2011a).

In vitro micronucleus assays

Pent-1-en-3-one was assayed for the induction of structural and numerical chromosomal aberrations in mammalian cells in vitro by examining the effect on the frequency of micronuclei in cultured human peripheral blood lymphocytes pooled from two healthy male donors both in the absence and in the presence of Aroclor-induced rat liver S9-mix. After stimulation with PHA for 48 h, cells were treated with pent-1-en-3-one either for 3 h (followed by 21 h of recovery) in the absence or in the presence of S9-mix, or for 24 h in the absence of S9-mix. A range-finding experiment had been conducted with and without S9-mix at 12 concentrations up to 841.2 μg/mL (10 mM). In the main assay, micronuclei were analysed at three concentrations for each treatment group. For 3-h treatment without S9-mix, the concentrations were 3.5, 4.25 and 4.75 μg/mL, for 3-h treatment with S9-mix the concentrations were 8.0, 12.0 and 16.0 μg/mL, and for 24-h treatment without S9-mix the concentrations were 3.0, 3.5 and 4.0 μg/mL. The levels of cytotoxicity (reduction in RI) analysed for micronucleus at the top concentrations reached 48% and 50% in the 3-h treatment in the presence of S9-mix and the 24-h treatment in the absence of S9-mix, respectively. Following a 3-h treatment in the absence of S9-mix, toxicity at the highest concentration (4.75 μg/mL) was only 38%. However, the toxicity curve was very steep and at the next higher concentration (5.0 μg/mL) toxicity was excessive (81% reduction in RI). One thousand binucleate cells per culture from two (or in some cases four) replicate cultures per concentration were scored for micronuclei.

Following the 3-h treatment without S9-mix, there was an increase in the frequency of MNBN cells from 0.2% in the solvent control to 0.55% at the lowest concentration. This increase was statistically significant at p < 0.05 but fell well within the historical control range and was therefore not considered to be biologically significant. There were no significant increases in MNBN frequency at the middle and high concentrations, and therefore no concentration-related response. In the presence of S9-mix, there were no statistically significant increases in mean MNBN cell frequency at any concentration. Following the 24-h treatment without S9-mix, a statistically significant (p < 0.01) increase in MNBN cell frequency from 0.40% in the control to 1.1% was observed at the maximum concentration of 4.0 μg/mL. This elevated frequency, which only just exceeded the historical control range for the laboratory (0–1.0%), was due entirely to an increase in MNBN frequency in only one of the two replicate cultures (the other replicate had a background MNBN frequency at a comparable level of toxicity), and the overall responses were not clearly concentration related. Thus, it seems most likely that the single replicate increase was due to chance. Treatment of the cells with pent-1-en-3-one under all conditions therefore resulted in frequencies of MNBN cells that were generally similar to those observed in concurrent and historical vehicle controls at all concentrations analysed (Lloyd, 2010). It was concluded by the applicant that pent-1-en-3-one did not induce micronuclei in cultured human peripheral blood lymphocytes when tested at toxic concentrations in both the absence and the presence of S9-mix (Lloyd, 2010).

The Panel noted that pent-1-en-3-one is extremely cytotoxic to human lymphocytes, with a very steep toxicity curve and therefore the substance can only be tested for genotoxicity in a narrow concentration range.

A summary of the in vitro genotoxicity data are given in Table 5.

2.4.2. In vivo data

No data available.
2.4.3. Conclusion by the CEF Panel in FGE.205

The two representative substances, oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], were both weakly genotoxic in bacteria with pent-1-en-3-one being the most potent (previously available data). In newly available data performed according to recent guidelines and Good Laboratory Practice (GLP), several studies were performed on oct-1-en-3-one in bacteria. In these assays, the test substance was highly cytotoxic with a steep toxicity curve, and there was a lack of reproducibility in the weak genotoxic response, which could be due to a slight day-to-day variation in the severity of the observed toxicity and a very narrow concentration range resulting in mutagenicity. Both substances were also tested in mammalian cells for gene mutations at the hprt locus and for structural and numerical chromosomal aberrations in the micronucleus assay. Also in mammalian cells, the test substances were highly cytotoxic. All the in vitro assays were well performed and each of the assays performed in mammalian cells were considered to be negative, when looking at them separately except the gene mutation assay with pent-1-en-3-one which was considered by the Panel to be equivocal, presumably due to severe cytotoxicity of the test compound. Due to positive effects in the bacterial mutagenicity assays of the two representative substances, which cannot be overruled by one negative and one equivocal gene mutation test in mammalian cells, an in vivo Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver is requested on the most potent substance, pent-1-en-3-one. As an alternative, a transgenic animal assay would also be acceptable.

3. Assessment

3.1. Additionally genotoxicity data considered by the Panel in FGE.205Rev1

In response to the data request in FGE.205, the Industry has submitted in vivo data on both pent-1-en-3-one [FL-no: 07.102] and oct-1-en-3-one [FL-no: 07.081] (Table 2).

Table 2: In vivo studies for the representative substances of FGE.205Rev1

<table>
<thead>
<tr>
<th>Substance</th>
<th>FL-no</th>
<th>In vivo study</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pent-1-en-3-one</td>
<td>07.102</td>
<td>Combined micronucleus (in bone marrow) and comet assay (in the liver and duodenum)</td>
<td>Keig-Shevlin (2015b,c)</td>
</tr>
<tr>
<td>Oct-1-en-3-one</td>
<td>07.081</td>
<td>Comet assay in the liver</td>
<td>Keig-Shevlin (2015a)</td>
</tr>
</tbody>
</table>

FGE: Flavouring Group Evaluation; FL-no: FLAVIS number.

Furthermore, to investigate the mechanism of action of the mutagenic activity seen in the bacterial reverse mutation tests by Beevers (2009 – described in Section 2.4.1), a new Ames test with oct-1-en-3-one was performed (Bowen, 2013). The study was performed according to OECD Guideline 471 (OECD, 1997), but not fully compliant with this guideline as only a single strain of bacteria was tested. The strain TA100 was chosen because mutagenicity was observed only in this strain (Beevers, 2009).

3.1.1. Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102]

Pent-1-en-3-one was tested for its potential to induce micronuclei in the polychromatic erythrocytes (PCE) of the bone marrow of treated rats and to induce DNA damage in the liver (Keig-Shevlin, 2015b) and duodenum (Keig-Shevlin, 2015c) of the same animals (Table 6).

Pent-1-en-3-one dissolved in corn oil was administered by gavage to male Han Wistar rats (six animals per dose group and 3 animals in the positive control group) at doses of 0, 10, 20 and 40 mg/kg body weight (bw) per day in three administrations at 0 (day 1), 24 (day 2) and 45 (day 3) h. The dose levels were identified in a dose-range finding study, in which morbidity and mortality have been observed at 60 mg/kg bw and higher doses and which demonstrated no gender differences. Accordingly, the authors of this study considered 40 mg/kg bw per day as the maximum tolerated dose (MTD). Ethyl methanesulfonate (EMS) was used as positive control, 150 mg/kg bw per day, dosed at 0, 24 and 45 h.

At day 3 (48 h) bone marrow (MN assessment), liver and duodenum (Comet analysis and histopathology) were sampled at necropsy, as well as blood for clinical chemistry.

Clinical chemistry showed that at 40 mg/kg bw per day there were decreases in alkaline phosphatase (ALP) activity (229 ± 70.4 IU/L in the negative control, 110 ± 16 IU/L at the highest
dose tested), total protein (55 ± 1.5 g/L in the negative control, 47 ± 2.6 g/L at the highest dose tested), albumin (36 ± 1 g/L in the negative control, 26 ± 1.5 g/L at the highest dose tested), albumin/globulin ratio (2 ± 0.12 in the negative control, 1.3 ± 0.23 at the highest dose tested), sodium (138 ± 1.6 mmol/L in the negative control, 133 ± 2.4 mmol/L at the highest dose tested) and calcium (2.64 ± 0.093 mmol/L in the negative control, 2.46 ± 0.071 mmol/L at the highest dose tested). There were no changes in either microscopic or macroscopic examinations which were related to the administration of pent-1-en-3-one. The Panel noted that the changes in clinical chemistry parameters cannot be considered as an indication of the liver toxicity, however, they might be due to general toxicity and could, in this case, be considered as an indirect indication of systemic availability. Accordingly, the negative outcome of this study can be considered valid.

3.1.1.1. Micronucleus arm of the assay

Animals treated with pent-1-en-3-one at all doses exhibited group mean %PCE that were either similar or demonstrated a slight reduction when compared to the concurrent vehicle control group and which were within the laboratory's historical vehicle control data, thus confirming there was no evidence of test article related bone marrow toxicity.

Animals displayed intragroup heterogeneity in the low-dose group (10 mg/kg bw per day). Therefore, the percentages of micronucleated polychromatic erythrocytes (MN PCE) in each treated group were compared with vehicle control using a Wilcoxon rank sum test. In addition, the Terpstra–Jonckheere test for dose response was performed.

Animals treated with pent-1-en-3-one at all doses exhibited MN PCE frequencies that were similar to the concurrent vehicle control group and which were considered consistent with the laboratory's historical vehicle control data. There were no statistically significant increases in micronucleus frequency for any of the groups receiving the test article, compared with the concurrent vehicle control (Keig-Shevlin, 2015b).

3.1.1.2. Comet arm of the assay

Liver

Following oral gavage administration of pent-1-en-3-one [FL-no: 07.102], there was no dose-related increase in the percentage of clouds in the liver, demonstrating that treatment with pent-1-en-3-one did not cause excessive liver toxicity which can interfere with Comet analysis.

Group mean percentage of tail intensity and tail moment values for all tested doses were similar to the concurrent vehicle control group. There was no evidence of any induction of DNA damage in cells isolated from the liver following treatment with pent-1-en-3-one (Keig-Shevlin, 2015b).

Duodenum

There was no dose-related increase in the percentage of clouds in duodenum cells following treatment with pent-1-en-3-one, thus demonstrating that treatment did not cause toxicity that could have interfered with Comet analysis.

Group mean percentage of tail intensity and tail moment values for all groups treated with pent-1-en-3-one were comparable with the group mean vehicle control data. There were no statistically significant differences in %tail intensity between treated and the vehicle control group. All individual animal data for tail intensity and tail moment, at all dose levels, were generally consistent with the vehicle control data and fell within the laboratory's historical control data, with the exception of 2 animals at the highest dose which fell below the historical control range observed for tail intensity. For one of these two animals, also the value of tail moment fell below the historical control range (Keig-Shevlin, 2015c).

3.1.1.3. Conclusion for pent-1-en-3-one

It is concluded that under the conditions of this study, pent-1-en-3-one [FL-no: 07.102], did not induce DNA damage in the liver and duodenum when male rats were dosed orally up to 40 mg/kg bw per day (an estimate of the MTD). Pent-1-en-3-one [FL-no: 07.102] did not increase the percentage of micronucleated PCE in bone marrow. The question whether or not the bone marrow was exposed is not relevant in this case because a previous in vitro micronucleus assay was negative and, accordingly, there was no need of follow-up with an in vivo micronucleus assay.
3.1.2. Oct-1-en-3-one (amyl vinyl ketone) [FL-no: 07.081]

Oct-1-en-3-one was tested in an Ames test (Bowen, 2013) in order to investigate the mechanism of action inducing mutagenicity in the bacterial reverse mutation tests by Beevers (2009 – described in Section 2.4.1). Oct-1-en-3-one was tested also through an in vivo Comet assay in rats in the liver (Keig-Shevlin, 2015a) for its potential to induce DNA damage (Tables 6 and 7).

3.1.2.1. Bacterial reverse mutation assay

Oct-1-en-3-one [FL-no: 07.081] has previously been tested for mutagenic effect in S. typhimurium TA100 (Beevers, 2009, see Section 2.4.1). A weak positive effect was observed, without consistent reproducibility, due to high toxicity of the test substance. A follow-up study has been submitted by industry (Bowen, 2013). In this study, oct-1-en-3-one (purity 98.4%) was tested in the Ames test (plate incorporation) at concentrations of 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 µg/plate, only in strain TA100 of S. typhimurium, in two separate experiments, in the presence and in the absence of metabolic activation, using triplicate plates. Negative and positive controls were included in quintuplicate and triplicate, respectively. In both experiments, oct-1-en-3-one was tested in the presence and in the absence of each of four distinct free radical/electrophile scavengers, in order to assess the role of the potential formation of reactive oxygen species in the mechanism inducing mutagenicity in strain TA100. The free radical/electrophile scavengers used were: glutathione at 500 µg/plate, N-acetyl cysteine at 500 µg/plate, catalase at 12.5 µg/plate and 2,5-dimethylfuran at 250 µg/plate (Table 7).

This study confirmed the high bacterial toxicity of oct-1-en-3-one. It induced cytotoxicity from concentrations of 125 and 250 µg/plate in the first and second experiment, respectively, without S9-mix, and from concentrations of 250 and 500 µg/plate with S9-mix. When the electrophile scavengers were added, there was a slight reduction in bacterial toxicity, but there was a difference in toxicity between the two experiments. In the absence of free radical/electrophile scavengers, oct-1-en-3-one increased the number of revertants, compared with the concurrent vehicle control, at least twofold or 1.5-fold, in the absence or in the presence of S9-mix, respectively, at 62.5 and 125 µg/plate.

There was no clear indication of the influence on the mutagenic effect of oct-1-en-3-one when the electrophile scavengers were added.

Overall, the applicant concluded that oct-1-en-3-one induced mutations in S. typhimurium TA100 under the conditions of the study, and that glutathione, N-acetyl cysteine and to a lesser extent 2,5-dimethylfuran reduced the mutagenic response. In contrary, catalase increased the mutagenic response in most cases. According to the applicant, these results indicate that the mutagenic effect is due to oxidative stress.

The Panel, however, noted that the results were not clearly reproducible and that the mutagenic responses were weak in all experiments. Therefore, the Panel concluded that no clear conclusion on the mechanism of mutagenicity induced by oct-1-en-3-one and by the other substances in this group can be drawn based on this study. The Panel noted that probably, the reduction in the number of revertants, observed in particular after the treatment with glutathione and N-acetyl cysteine, is due to a reduction in bacterial toxicity. In addition, strain TA102 is more sensitive to oxidative stress than TA100, but oct-1-en-3-one was negative in strain TA102 in a previous mutagenicity test. The Panel considered that the newly submitted study (Bowen, 2013) does not contribute in elucidating the mechanism of genotoxicity of oct-1-en-3-one [FL-no: 07.081].

3.1.2.2. Comet assay

Oct-1-en-3-one [FL-no: 07.081] dissolved in corn oil was administered by gavage to male Han Wistar rats (six animals per dose group and three animals in the positive control group) at doses of 0, 45, 90 and 180 mg/kg bw per day in two administrations at 0 (day 1) and 21 (day 2) h. The dose levels were identified in a dose-range finding study, which also demonstrated no gender differences. EMS was used as positive control, 200 mg/kg bw, in a single administration at 21 h (day 2).

At day 2 (24 h), liver and duodenum were sampled at necropsy, as well as blood and tissue for clinical chemistry and histopathology. The duodenum, however, was not analysed in the comet assay. There were no findings either in clinical chemistry, microscopic or macroscopic examinations which were related to the administration of oct-1-en-3-one.

There was no dose-related increase in clouds percentage in the liver following treatment with oct-1-en-3-one, thus demonstrating that treatment with oct-1-en-3-one did not cause excessive toxicity, which can interfere with Comet analysis, following oral gavage administration.
Following treatment with oct-1-en-3-one at all dose levels, group mean %tail intensity and tail moment values for all tissues were similar to the concurrent vehicle control group. There was no evidence of any induction of DNA damage in cells isolated from the liver following treatment with oct-1-en-3-one.

The Panel noted that all group mean values for %tail intensity and tail moment were very low including the positive control that was below the historical control range. Therefore, the Panel considered this study to be of limited validity.

3.1.2.3. Conclusion for oct-1-en-3-one

Oct-1-en-3-one [FL-no: 07.081] induced gene mutations in *S. typhimurium* strain TA100 in the presence and in the absence of S9-mix. It was negative in the hpert assay and in the *in vitro* micronucleus assay (EFSA CEF Panel, 2012). New available data confirm the high toxicity of oct-1-en-3-one and its mutagenicity on strain TA100.

The bacterial reverse mutation assay on TA100 in the presence of free radical/electrophile scavengers does not allow to elucidate the mechanism of gene mutations induced by oct-1-en-3-one. The *in vivo* comet assay in the liver did not provide evidence of any induction of DNA damage in the liver, however, this study is considered of limited validity.

4. Overall conclusion

Due to positive effects in the bacterial mutagenicity assays of the two representative substances pent-1-en-3-one [FL-no: 07.102] and oct-1-en-3-one [FL-no: 07.081], an *in vivo* Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver was requested on the most potent substance, pent-1-en-3-one (EFSA CEF Panel, 2012).

In response to the data request in FGE.205, the industry has submitted *in vivo* data on both pent-1-en-3-one and oct-1-en-3-one. Pent-1-en-3-one [FL-no: 07.102] was tested for its potential to induce micronuclei in the PCE of the bone marrow of treated rats and to induce DNA damage in the liver and duodenum of the same animals. Oct-1-en-3-one [FL-no: 07.081], was tested, in a Comet assay, for its potential to induce DNA damage in the liver of rats. Furthermore, to investigate the mechanism of action of the mutagenic activity observed in the bacterial reverse mutation tests of previous studies, a new Ames test with oct-1-en-3-one was performed with strain TA100.

Pent-1-en-3-one [FL-no: 07.102] tested *in vivo* in a combined micronucleus and comet assay did not show genotoxic effects in either the liver or duodenum of treated rats. The negative results of the bone marrow micronucleus assay are considered inconclusive because there is no evidence of bone marrow exposure to the tested substance. However, as results of the *in vitro* micronucleus assay were negative, no additional *in vivo* follow-up studies on clastogenicity and aneugenicity were needed.

The bacterial mutation assay provided for oct-1-en-3-one [FL-no: 07.081] confirms the weak mutagenic effect in bacteria shown in previous studies, but does not clarify the mechanism of action. The liver comet assay is considered of limited validity due to low values of mean tail intensity and tail moment. However, based on the data available on the most potent of the two representative substances for subgroup 1.2.2, pent-1-en-3-one [FL-no: 07.102], the Panel concluded that there is no concern for genotoxicity and accordingly all the 13 substances in subgroup 1.2.2 [FL-no: 02.023, 02.099, 02.104, 02.131, 02.136, 02.155, 02.187, 07.081, 07.102, 07.161, 07.210, 09.281 and 09.282] can be evaluated using the Procedure.
Summary of specifications

Table 3: Summary of specifications for the substances in the Flavouring Group Evaluation 205 (JECFA, 2002, 2009)

<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(a)</th>
<th>Solubility in ethanol(b)</th>
<th>Boiling point, °C(c)</th>
<th>Melting point, °C</th>
<th>ID test</th>
<th>Assay minimum</th>
<th>Refrac. index(d)</th>
<th>Spec. gravity(e)</th>
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<tr>
<td>02.023</td>
<td>1152</td>
<td>Oct-1-en-3-ol</td>
<td></td>
<td>2805</td>
<td>72</td>
<td>3391-86-4</td>
<td>Liquid</td>
<td>C₈H₁₆O</td>
<td>128.22</td>
<td>Insoluble</td>
<td>Miscible</td>
<td>175 - 175.2</td>
<td>NMR 96%</td>
<td></td>
<td></td>
<td>1.431 - 1.442</td>
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<td>Pent-1-en-3-ol</td>
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<td>C₅H₁₀O</td>
<td>86.13</td>
<td>Sparingly soluble</td>
<td>Miscible</td>
<td>114</td>
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<td>Hex-1-en-3-ol</td>
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<td>Insoluble</td>
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<td>133.5 - 134</td>
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<td>C₄H₈O</td>
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<td>Miscible</td>
<td>215</td>
<td>NMR MS 97%</td>
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<td></td>
<td>1.439 - 1.446</td>
<td>0.836 - 0.842</td>
</tr>
<tr>
<td>02.155</td>
<td>1842</td>
<td>1-Hepten-3-ol</td>
<td></td>
<td>4129</td>
<td>10218</td>
<td>4938-52-7</td>
<td>Liquid</td>
<td>C₇H₁₄O</td>
<td>114.19</td>
<td>Practically insoluble or insoluble</td>
<td>Miscible</td>
<td>155</td>
<td>MS 97%</td>
<td></td>
<td></td>
<td>1.431 - 1.437</td>
<td>0.834 - 0.837</td>
</tr>
<tr>
<td>02.187</td>
<td></td>
<td>Non-1-en-3-ol</td>
<td></td>
<td>10291</td>
<td>21964</td>
<td>44-3</td>
<td>Liquid</td>
<td>C₉H₁₈O</td>
<td>142.24</td>
<td>Practically insoluble or insoluble</td>
<td>Freely soluble</td>
<td>195</td>
<td>MS 98%</td>
<td></td>
<td></td>
<td>1.438 - 1.444</td>
<td>0.835 - 0.845</td>
</tr>
<tr>
<td>07.081</td>
<td>1148</td>
<td>Oct-1-en-3-one</td>
<td></td>
<td>3515</td>
<td>2312</td>
<td>4312-99-6</td>
<td>Liquid</td>
<td>C₈H₁₄O</td>
<td>126.20</td>
<td>Insoluble</td>
<td>Miscible</td>
<td>37 - 38 (3 hPa)</td>
<td>NMR 96%</td>
<td></td>
<td></td>
<td>1.428 - 1.439</td>
<td>0.813 - 0.819</td>
</tr>
<tr>
<td>07.102</td>
<td>1147</td>
<td>Pent-1-en-3-one</td>
<td></td>
<td>3382</td>
<td>11179</td>
<td>1629-58-9</td>
<td>Liquid</td>
<td>C₁₀H₁₈O</td>
<td>84.12</td>
<td>Insoluble</td>
<td>Miscible</td>
<td>68 - 70 (260 hPa)</td>
<td>NMR 97%</td>
<td></td>
<td></td>
<td>1.417 - 1.422</td>
<td>0.842 - 0.848</td>
</tr>
<tr>
<td>07.161</td>
<td></td>
<td>Hex-1-en-3-one</td>
<td></td>
<td>1629-60-3</td>
<td></td>
<td></td>
<td>Liquid</td>
<td>C₁₀H₁₈O</td>
<td>98.14</td>
<td>Practically insoluble or insoluble</td>
<td>Freely soluble</td>
<td>128</td>
<td>MS 95%</td>
<td></td>
<td></td>
<td>1.420 - 1.426</td>
<td>0.849 - 0.855</td>
</tr>
<tr>
<td>07.210</td>
<td></td>
<td>1-Nonene-3-one</td>
<td></td>
<td>24415-26-7</td>
<td></td>
<td></td>
<td>Liquid</td>
<td>C₁₀H₁₈O</td>
<td>140.22</td>
<td>Insoluble</td>
<td>Freely soluble</td>
<td>80 (16 hPa)</td>
<td>MS 95%</td>
<td></td>
<td></td>
<td>1.436 - 1.442</td>
<td>0.826 - 0.830</td>
</tr>
</tbody>
</table>
### Summary of safety evaluation of the JECFA substances in FGE.205

Table 4: Current safety evaluation status applying the Procedure, based on the MSDI approach, (JECFA, 2002, 2009)

<table>
<thead>
<tr>
<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>EU MSDI(a) (µg/capita per day)</th>
<th>Class(b) Evaluation procedure(c)</th>
<th>JECFA outcome on the named compound(d or e)</th>
<th>EFSA conclusion for genotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.023</td>
<td>1152</td>
<td>Oct-1-en-3-ol</td>
<td></td>
<td>240</td>
<td>Class III A3: intake below threshold</td>
<td>Genotoxicity concern could be ruled out</td>
<td></td>
</tr>
<tr>
<td>02.099</td>
<td>1150</td>
<td>Pent-1-en-3-ol</td>
<td></td>
<td>2.1</td>
<td>Class II A3: intake below threshold</td>
<td>Genotoxicity concern could be ruled out</td>
<td></td>
</tr>
<tr>
<td>02.104</td>
<td>1151</td>
<td>Hex-1-en-3-ol</td>
<td></td>
<td>0.55</td>
<td>Class II A3: intake below threshold</td>
<td>Genotoxicity concern could be ruled out</td>
<td></td>
</tr>
<tr>
<td>02.131</td>
<td></td>
<td>But-3-en-2-ol</td>
<td></td>
<td>0.0012</td>
<td>Class II No evaluation</td>
<td>Not evaluated by JECFA</td>
<td></td>
</tr>
<tr>
<td>02.136</td>
<td>1153</td>
<td>Dec-1-en-3-ol</td>
<td></td>
<td>ND</td>
<td>Class II A3: intake below threshold</td>
<td>Genotoxicity concern could be ruled out</td>
<td></td>
</tr>
<tr>
<td>02.155</td>
<td>1842</td>
<td>1-Hepten-3-ol</td>
<td></td>
<td>0.13</td>
<td>Class II A3: intake below threshold</td>
<td>Genotoxicity concern could be ruled out</td>
<td></td>
</tr>
<tr>
<td>02.187</td>
<td></td>
<td>Non-1-en-3-ol</td>
<td></td>
<td>0.58</td>
<td>Class II No evaluation</td>
<td>Not evaluated by JECFA</td>
<td></td>
</tr>
</tbody>
</table>
### Genotoxicity data considered by the Panel in FGE.205

Table 5: Summary of genotoxicity data considered by the Panel in FGE.205

<table>
<thead>
<tr>
<th>FL-no</th>
<th>Chemical name</th>
<th>Test system in vitro</th>
<th>Test object</th>
<th>Concentrations tested and test conditions</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.102</td>
<td>Pent-1-en-3-one</td>
<td>Reverse mutation</td>
<td>Salmonella typhimurium TA100</td>
<td>0–168.33 µg/plate(^{(a,b,c)})</td>
<td>Positive</td>
<td>Deininger et al. (1990)</td>
<td>Highly toxic especially without S9. Mutagenicity observed with and without S9. Reduced mutagenicity with inhibition of monooxygenase, enhanced mutagenicity with addition of epoxide hydrolase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not reported</td>
<td>Positive</td>
<td>Eder et al. (1993)</td>
<td>Assay conditions, doses and control revertants were not reported but same data as in Deininger et al. (1990)</td>
</tr>
</tbody>
</table>

**Notes:**
- **FL-no:** FLAVIS number
- **EU Register name:**
- **Structural formula:**
- **EU MSDI\(^{(a)}\)**: Maximised survey-derived daily intake (µg/capita per day)
- **US MSDI:**
- **Class\(^{(b)}\):**
- **Evaluation procedure\(^{(c)}\):**
- **JECFA outcome on the named compound\(^{(d \text{ or } e)}\):**
- **EFSA conclusion for genotoxicity:**
- **JECFA:** The Joint FAO/WHO Expert Committee on Food Additives
- **FGE:** Flavouring Group Evaluation
- **FL:** FLAVIS number
- **EU:** European Union
- **MSDI:** maximised survey-derived daily intake
- **EFSA:** European Food Safety Authority
- **ND:** Not determined

\(^{(a)}\): EU MSDI: Amount added to food as flavour in (kg/\text{year}) \times 10^{9}/0.1 \times \text{population in Europe} (= 375 \times 10^{6}) = \mu g/\text{capita per day.}

\(^{(b)}\): Thresholds of concern: Class I = 1,800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.

\(^{(c)}\): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

\(^{(d)}\): No safety concern based on intake calculated by the MSDI approach of the named compound.

\(^{(e)}\): Data must be available on the substance or closely related substances to perform a safety evaluation.
<table>
<thead>
<tr>
<th>FL-no</th>
<th>Chemical name</th>
<th>Test system in vitro</th>
<th>Test object</th>
<th>Concentrations tested and test conditions</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SOS chromotest</td>
<td><em>Escherichia coli</em> PQ37</td>
<td>0–5.05 μg(^{a})</td>
<td>Positive</td>
<td>Deininger et al. (1990)</td>
<td>Highly toxic. Positive in the presence of S9-mix (induction factor of 1.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0–8.41 μg(^{a})</td>
<td>Positive</td>
<td>Eder et al. (1991)</td>
<td>Reported in graphical form for methyl vinyl ketone only, but patterns were noted to be similar for pent-1-en-3-one, previously published by Deininger et al. (1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0–8.41 μg(^{a})</td>
<td>Positive</td>
<td>Eder et al. (1993)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNA adducts</td>
<td></td>
<td><em>E. coli</em> PQ37</td>
<td>168 mg</td>
<td>Positive</td>
<td>Eder et al. (1993)</td>
<td>1,(N^2)-cyclic deoxyguanosine adducts and 7-linear guanine adducts were isolated</td>
</tr>
<tr>
<td></td>
<td><em>hppt</em> assay</td>
<td></td>
<td>Mouse lymphoma L5178Y cells</td>
<td>0.1–1.2 μg/mL(^{d,j})</td>
<td>Equivocal</td>
<td>Lloyd (2011a)</td>
<td>Highly toxic. Could only be tested in a narrow concentration range. Some indication of genotoxic effect at toxic concentrations, which was not reproducible</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Micronucleus induction</td>
<td>Human peripheral blood lymphocytes</td>
<td>3.50–4.75 μg/mL(^{d,g})</td>
<td>Negative</td>
<td>Lloyd (2010)</td>
<td>Highly toxic. Could only be tested in a narrow concentration range. Some indication of genotoxic effect at toxic concentrations, which was not reproducible</td>
</tr>
<tr>
<td>[07.081] Oct-1-en-3-one</td>
<td>Reverse Mutation</td>
<td><em>S. typhimurium</em> TA98, TA100, TA1535, TA1537 and TA102</td>
<td></td>
<td>0.32–1,000 μg/plate(^{b})</td>
<td>Positive</td>
<td>Beevers (2009)</td>
<td>All strains were negative except TA100, with S9-mix treatment at 200 μg/plate resulted in 4.6-fold increase in revertants. Toxicity was observed at 1,000 μg/plate</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. typhimurium</em> TA98, TA100, TA1535, TA1537 and TA102</td>
<td></td>
<td>15.6–500 μg/plate(^{b,c,i})</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. typhimurium</em> TA100</td>
<td></td>
<td>50–500 μg/plate(^{c,f})</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. typhimurium</em> TA100</td>
<td></td>
<td>100–300 μg/plate(^{b,c})</td>
<td>Positive</td>
<td></td>
<td>The plate incorporation method resulted in increased revertants by 2.3- to 3-fold with a 125–200 μg/plate treatment in the absence of S9-mix and 2.2- to 2.9-fold with a 100–200 μg/plate treatment in the presence of S9-mix, neither being dose dependent. The only preincubation treatment that resulted in increased revertants was 45 μg/plate treatments in the presence of S9-mix (1.7-fold)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15–120 μg/plate(^{f,i})</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Summary of genotoxicity data considered by the panel in FGE.205Rev1

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Test system in vitro</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>Pent-1-en-3-one [07.102]</td>
<td>Micronucleus assay</td>
<td>Han Wistar Rat; M</td>
<td>Gavage</td>
<td>0, 10, 20 and 40 mg/kg bw per day</td>
<td>Negative</td>
<td>Keig-Shevlin (2015b,c)</td>
<td>Study performed in accordance with OECD guideline 474 and GLP. No proof of bone marrow exposure.</td>
</tr>
<tr>
<td></td>
<td>Comet assay</td>
<td>Han Wistar Rat; M</td>
<td>Gavage</td>
<td></td>
<td>Negative&lt;sup&gt;(a,b)&lt;/sup&gt;</td>
<td></td>
<td>Study performed in accordance with OECD guideline 489 and GLP.</td>
</tr>
<tr>
<td>Oct-1-en-3-one [07.081]</td>
<td>Comet assay</td>
<td>Han Wistar Rat; M</td>
<td>Gavage</td>
<td>0, 45, 90 and 180 mg/kg bw per day</td>
<td>Inconclusive&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Keig-Shevlin (2015a)</td>
<td>Study performed in accordance with GLP and internationally recognised protocols available before the publication of OECD guideline 489. The study was considered of limited validity.</td>
</tr>
</tbody>
</table>

FGE: Flavouring Group Evaluation; FL-no: FLAVIS number; bw: body weight; OECD: Organisation for Economic Co-operation and Development; GLP: Good Laboratory Practice.

(a): Scored in liver cells.

(b): Scored in duodenum cells.
Table 7: In vitro study to investigate the mechanism of mutagenicity induced by oct-1-en-3-one in S. typhimurium strain TA100

<table>
<thead>
<tr>
<th>Chemical name [FL-no]</th>
<th>Test system in vitro</th>
<th>Test object</th>
<th>Concentrations tested and test conditions</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
</table>

FL-no: FLAVIS number.
(a): With and without metabolic activation.
(b): The following free radical/electrophile scavengers were added: glutathione, N-acetyl cysteine, catalase, 2,5-dimethylfuran.

Documentation provided to EFSA

5) EFFA (European Flavour Association), 2011. Submission by the European Flavour Association to the European Food Safety Authority. Flavouring Group Evaluation 19 Subgroup 1.2.2 (corresponding to FGE.205): Submission of additional data related to FGE.19 subgroup 1.2.2. 16 December 2011. FLAVIS/8.138.


References

Abbreviations
ALP alkaline phosphatase
bw body weight
CAS Chemical Abstracts Service
CEF Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE Council of Europe
EFFA European Flavour Association
EMS ethyl methanesulfonate
FAO Food and Agriculture Organization of the United Nations
FEMA Flavor and Extract Manufacturers Association
FGE Flavouring Group Evaluation
FLAVIS Flavour Information System database
GLP Good Laboratory Practice
HPRT hypoxanthine-guanine phosphoribosyl transferase
ID identity
IR infrared
JECFA The Joint FAO/WHO Expert Committee on Food Additives
MF mutant frequency
MN micronucleus
MNBN micronucleated binucleated (cells)
MN PCE micronucleated polychromatic erythrocytes
MS mass spectrometry
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSDI</td>
<td>maximised survey-derived daily intake</td>
</tr>
<tr>
<td>MTD</td>
<td>maximum tolerated dose</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>PCE</td>
<td>polychromatic erythrocytes</td>
</tr>
<tr>
<td>PHA</td>
<td>phytohaemagglutinin</td>
</tr>
<tr>
<td>(Q)SAR</td>
<td>(quantitative) structure–activity relationship</td>
</tr>
<tr>
<td>RI</td>
<td>replication index</td>
</tr>
<tr>
<td>RS</td>
<td>relative survival</td>
</tr>
<tr>
<td>TCPO</td>
<td>trichloropropene oxide</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>