

Scientific Opinion

Scientific Opinion on Flavouring Group Evaluation 205 (FGE.205):

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 by EFSA¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider in the Flavouring Group Evaluation 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 and the Panel recommend that an *in vivo* Comet assay on the first site of contact (e.g. the stomach) and on the liver is requested on the most potent of the representative substances, pent-1-en-3-one.

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KEY WORDS

α,β -unsaturated ketones, aliphatic ketones, terminal double-bond, flavouring substances, safety evaluation.

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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.

The present Flavouring Group Evaluation 205 (FGE.205), corresponding to subgroup 1.2.2 of FGE.19, concerns four α,β -unsaturated aliphatic ketones with a terminal double-bond and nine precursors for such ketones. The 13 substances under consideration in the present evaluation are α,β -unsaturated ketone structures or can be metabolised to such, which are considered to be structural alerts for genotoxicity 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, 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 *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 and the Panel recommends that an *in vivo* Comet assay on the first site of contact (e.g. the stomach) and on the liver is requested on the most potent of the representative substances, pent-1-en-3-one.

TABLE OF CONTENTS

| | |
|--|----|
| Abstract | 1 |
| Summary | 2 |
| Table of contents | 3 |
| Background | 4 |
| Terms of reference as provided by the Commission | 5 |
| Assessment | 5 |
| 1. Presentation of the substances in the Flavouring Group..... | 5 |
| 1.1. Description..... | 5 |
| 1.2. Representative substances for subgroup 1.2.2 | 5 |
| 2. Additionally submitted genotoxicity data on representative substances of subgroup 1.2.2 | 6 |
| 2.1. <i>In vitro</i> data | 6 |
| 2.1.1. Oct-1-en-3-one (amyl vinyl ketone) [FL-no: 07.081] | 6 |
| 2.1.2. Pent-1-en-3-one (ethyl vinyl ketone) [FL-no: 07.102] | 9 |
| 2.2. <i>In vivo</i> data..... | 11 |
| 3. Conclusion by the CEF Panel..... | 11 |
| Specification Summary of the Substances in the Flavouring Group Evaluation 205 (JECFA, 2002d; JECFA, 2009b)..... | 12 |
| Genotoxicity (<i>in vitro</i>)..... | 16 |
| References | 19 |
| abbreviations | 21 |

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) 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, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). 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, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

After the completion of the evaluation programme the Union list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

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, 2008b).

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, 2008b). 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. 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 Flavouring Group Evaluations (FGEs) were established, FGE.200, 204, 205, 206, 207, 208, 209, 211, 215, 219, 221, 222, 223, 224 and 225).

For 11 subgroups the Panel decided, based on the available genotoxicity data and (Q)SAR predictions, that a further scrutiny of the data should take place before requesting additional data from the Flavouring Industry on genotoxicity. These subgroups were evaluated in FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220. For the substances in FGE.202, 214 and 218 it was concluded that a genotoxic potential could be ruled out and accordingly these substances will be evaluated using the

Procedure. For all or some of the substances in the remaining FGEs, FGE.201, 203, 210, 212, 213, 216, 217 and 220 the genotoxic potential could not be ruled out.

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

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.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the following 13 substances: oct-1-en-3-ol [FL-no: 02.023], pent-1-en-3-ol [FL-no: 02.099], hex-1-en-3-ol [FL-no: 02.104], but-3-en-2-ol [FL-no: 02.131], dec-1-en-3-ol [FL-no: 02.136], 1-hepten-3-ol [FL-no: 02.155], non-1-en-3-ol [FL-no: 02.187], oct-1-en-3-one [FL-no: 07.081], pent-1-en-3-one [FL-no: 07.102], hex-1-en-3-one [FL-no: 07.161], 1-nonene-3-one [FL-no: 07.210], oct-1-en-3-yl acetate [FL-no: 09.281] and oct-1-en-3-yl butyrate [FL-no: 09.282], in accordance with Commission Regulation (EC) N° 1565/2000.

ASSESSMENT

1. Presentation of the substances in the Flavouring Group

1.1. Description

The present Flavouring Group Evaluation 205 (FGE.205), corresponding to subgroup 1.2.2 of FGE.19, concerns four α,β -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 1.

Nine of the 13 substances have previously been evaluated by the JECFA at their 59th and 69th meetings (JECFA, 2002c; JECFA, 2009c). A summary of their current evaluation status by the JECFA and the outcome of this consideration is presented in Table 2.

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

1.2. Representative substances for subgroup 1.2.2

The Panel has identified two substances in subgroup 1.2.2 which will represent the other 11 substances in this subgroup (EFSA, 2008bc). For these two substances genotoxicity data according to the test strategy (EFSA, 2008bb) have been requested. The representative substances are listed in Table 1.2.

Table 1.2 Representative substances for subgroup 1.2.2 of FGE.19

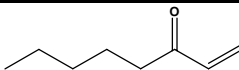
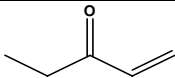
| FL-no JECFA- no | Subgroup | EU Register name | Structural formula | FEMA no CoE no CAS no |
|-----------------------|----------|------------------|--|-----------------------------|
| 07.081 1148 | 1.2.2 | Oct-1-en-3-one |  | 3515 2312 4312-99-6 |

Table 1.2 Representative substances for subgroup 1.2.2 of FGE.19

| FL-no JECFA- no | Subgroup | EU Register name | Structural formula | FEMA no CoE no CAS no |
|-----------------------|----------|------------------|--|-----------------------------|
| 07.102 1147 | 1.2.2 | Pent-1-en-3-one |  | 3382 11179 1629-58-9 |

2. Additionally submitted genotoxicity data on representative substances of subgroup 1.2.2

The Industry has submitted data concerning genotoxicity studies (EFFA, 2011p) for the two representative substances for 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].

2.1. *In vitro* data

An Ames test, a *hprt* assay and an *in vitro* micronucleus assay has been performed with oct-1-en-3-one [FL-no: 07.081]. An *in vitro* micronucleus assay and a *hprt* assay has 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, 2008b) with pent-1-en-3-one were included in the submission. An overview of the studies are summarised in Table 3.

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

2.1.1.1. Genotoxicity in bacteria

An Ames assay was conducted 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 presence of S9-mix in strain TA100 only, using final concentrations of oct-1-en-3-one at 1.6, 8, 40, 200, 1000 and 5000 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 1000 µg/plate. Following these treatments, evidence of toxicity was observed in all strains at concentrations of 200 and/or 1000 µg/plate, both in the absence and in the presence of S9-mix. 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 1000 µg/plate (Beevers, 2009c).

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 pre-incubation 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 2-fold increase threshold. In addition to the pre-incubation 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 since 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. Pre-incubation 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 (pre-incubation 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 pre-incubation 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.

2.1.1.2. *hprt* assays

In 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 were conducted for 3 hours in the absence and presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial fraction (S9-mix), followed by a third experiment conducted for 24 hours in the absence of S9-mix. In the cytotoxicity range-finding experiment using a 3-hour treatment, ten concentrations were tested in the absence and presence of S9-mix, ranging from 2.5 to 1262 µg/mL (10 mM). The highest concentrations not resulting in severe cytotoxicity were 2.5 µg/mL in the absence of S9-mix and 4.9 µg/mL in the presence of S9-mix, which gave 2 % and 9 % relative survival (RS), respectively. In the cytotoxicity range-finding experiment using 24-hour treatments, nine concentrations were tested in the absence of S9-mix, ranging from 0.01 to 3 µg/mL. The highest concentration to provide > 10 % RS was 1.5 µg/mL, which gave 22 % RS. Based on this range finding study, twelve concentrations from 0.05 to 2.5 µg/mL in the absence of S9-mix, and from 0.5 to 7.5 µg/mL in the presence of S9-mix, were tested in the first mutation experiment using 3-hour treatments, and 7 days recovery period. The highest non-toxic concentrations were 2 µg/mL in the absence of S9-mix and 3 µg/mL in the presence of S9-mix, which gave 12 % and 19 % RS, respectively. In the absence of S9-mix, a statistically significant increase in mutant frequency (MF; 4.23 mutants per 10⁶ viable cells compared to the mean vehicle control MF of 1.10 mutants per 10⁶ viable cells) was observed at the highest concentration (2 µg/mL), but there was no statistically significant linear trend (Lloyd, 2011c).

In the second experiment using 3-hour treatments, ten concentrations ranging from 0.5 to 3 µg/mL in the absence of S9-mix, and from 0.5 to 4.5 µg/mL in the presence of S9-mix, were tested. Since the highest concentration evaluated in the first experiment (2 µg/mL) resulted in a relative survival < 10

%, the highest concentration evaluated in the second experiment was 1.8 µg/mL in the absence of S9-mix (11 % RS) and 4.5 µg/mL in the presence of S9-mix (24 % RS). There were no statistically significant increases in MF at any concentration analyzed 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-hour treatment in the absence of S9-mix, eleven concentrations ranging from 0.1 to 2.5 µg/mL were tested. Seven days after treatment the highest concentration not too toxic for selection (1.2 µg/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 µg/mL) and 3 hours treatment, which however could not be reproduced in the second experiment using the same treatment conditions and a third experiment using 24 hours treatment. It is therefore concluded, that this gene mutation test is negative under the experimental conditions performed.

2.1.1.3. *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 presence of metabolic activation (S9-mix).

After stimulation for 48 hours with phytohaemagglutinin (PHA) cells were treated with oct-1-en-3-one for 3 hours (followed by 21 hours recovery) with and without S9-mix and for 24 hours without S9-mix. An initial range-finding experiment using 4.6 - 1262 µg/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 - 20 µg/mL (absence of S9-mix) or 5 - 40 µg/mL (presence of S9-mix) were chosen for the main experiment with 3-hour treatments. The 3+21 hours treatment in the absence of S9-mix resulted in 81 - 99 % toxicity at 10 µg/mL and above. Concentrations of 2, 4, and 8 µg/mL resulted in 0.20 - 0.35 % MNBN cell frequency which was below that of the concurrent control. The 3+21 hours treatment in the presence of S9-mix resulted in 81 % cytotoxicity or greater at concentrations of 20 µg/mL and above. Five, 10 and 15 µg/mL treatments resulted in MNBN cell frequencies (0.3 - 0.55 %) similar to that of the control (0.5 %) (Lloyd, 2011b).

Since the 3-hour treatments produced negative results for micronucleus (MN) induction, treatments were also performed over a 24-hour period in the absence of S9. In the range-finding experiment concentrations again ranged up to 1262 µg/mL (10 mM) but cells did not replicate (zero RI) at concentrations of 35.33 µg/mL and above. Also there was 94 % toxicity at 12 µg/mL. Therefore, 10 µg/mL was chosen as the top concentration for evaluation (range of tested concentrations are tabulated in Table 3), where RI was reduced by 62 %. Treatment at this concentration resulted in a statistically significant ($p < 0.01$) increase in mean MNBN (micronucleated binucleated cells) cell frequency to 1.65 % (concurrent control was 0.65 %). However, the MNBN frequency only exceeded the normal range in 1 of the 2 replicate cultures, and there was no increase in MNBN frequency at 8 µg/mL where reduction in RI was 58 %, or any of the lower concentrations. Given that the only positive response was in 1 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 hours in the absence and presence of S9-mix and for 24+0 hours in the absence of S9-mix (Lloyd, 2011b).

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

2.1.2.1. Genotoxicity in bacteria

Previously available in vitro data

In a study by Deininger et al. (Deininger et al., 1990), ethyl vinyl ketone (EVK) was tested for the induction of gene mutations in *S. typhimurium* strain TA100 both with and without Aroclor induced rat liver S9-mix. The pre-incubation 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 1293 revertants per µmol without S9-mix and 748 revertants per µmol with S9-mix. Though the peak revertant rate was higher with S9-mix (1250 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 monooxygenase) the mutagenic response disappeared completely, whereas an addition of TCPO (an inhibitor of epoxide hydrolase) resulted in an increase of mutagenic activity, indicating that epoxidation of the double bond by S9-mix could also play a role in the mutagenicity of EVK. 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 characterized guanine and deoxyguanosin adducts with EVK, indicating that EVK can form DNA adducts, which can give rise to mutations (Eder et al., 1991a; Eder et al., 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., 1991a; Eder et al., 1993). Ethyl vinyl ketone 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.

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 hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus.

2.1.2.2. *hprt* assays

Induction of forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus after treatment with pent-1-en-3-one in the absence and 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 hours 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 (range of tested concentrations are tabulated in Table 3). Percent 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 mutant frequency 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 mutant frequency 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 mutant frequency 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 mutant frequency after (again) 3-hour treatment with pent-1-en-3-one in the presence of S9-mix but also after 24-hour treatment in the absence of S9-mix (2 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 mutant frequency at any concentration analyzed 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 treatment conditions even at higher and more toxic concentrations (the maximum concentrations analyzed were 5 and 7 µg/mL in the first and second experiments, respectively). Following 24-hour treatments in the absence of S9-mix the top concentrations evaluated (1.0 and 0.8 µg/ml in the 2 separate experiments) reduced RS to 12 % in each case, and therefore achieved the required level of toxicity for a robust test (range of tested concentrations are tabulated in Table 3). There were no statistically significant increases in mutant frequency at any concentration and no significant linear trend in either of the two 24-hour 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 hours treatment. Although, this positive effect was not reproduced in a second experiment using the same experimental design and a third experiment using 24 hours treatment without S9-mix, it was concluded by the Panel that the results in this *in vitro* gene mutation test is equivocal (Lloyd, 2011a).

2.1.2.3. *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 2 healthy male donors both in the absence and presence of Aroclor induced rat liver S9-mix. After stimulation with PHA for 48 hours cells were treated with pent-1-en-3-one either for 3 hours (followed by 21 hours of recovery) in the absence or presence of S9-mix, or for 24 hours 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 analyzed at three concentrations for each treatment group. For 3-hour treatment without S9-mix the concentrations were 3.5, 4.25 and 4.75 µg/ml, for 3-hour treatment with S9-mix the concentrations were 8.0, 12.0 and 16.0 µg/ml, and for 24-hour treatment without S9-mix the concentrations were 3.0, 3.5 and 4.0 µg/ml. The levels of cytotoxicity (reduction in replication index, RI) analysed for micronucleus at the top concentrations reached 48 and 50 % in the 3-hour treatment in the presence of S9-mix and the 24-hour treatment in the absence of S9-mix respectively. Following a 3-hour 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 2 (or in some cases 4) replicate cultures per concentration were scored for micronuclei.

Following the 3-hour treatment without S9-mix, there was an increase in the frequency of micronucleated binucleate cells (MNBN) 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-hour 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 1 of the 2 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, 2010b). 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 presence of S9-mix (Lloyd, 2010b).

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 3.

2.2. *In vivo* data

No data submitted

3. Conclusion by the CEF Panel

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 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 of 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.

SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 205 (JECFA, 2002D; JECFA, 2009B)

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

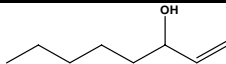
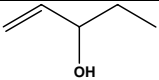
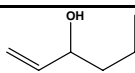
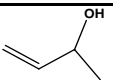
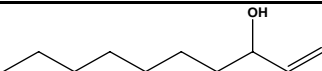
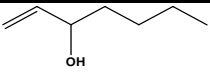
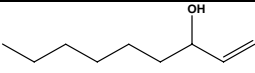
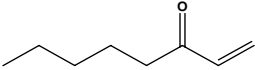
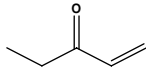
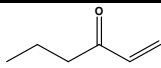
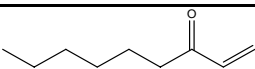
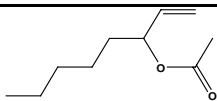
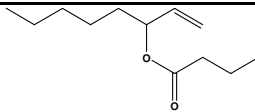
| FL-no JECFA- no | EU Register name | Structural formula | FEMA no CoE no CAS no | Phys.form Mol.formul a Mol.weight | Solubility 1) Solubility in ethanol 2) | Boiling point, °C 3) Melting point, °C ID test Assay minimum | Refrac. Index 4) Spec.gravity 5) |
|-----------------------|-------------------|---|-----------------------------|---|---|--|--|
| 02.023 1152 | Oct-1-en-3-ol 6) |  | 2805 72 3391-86-4 | Liquid C ₈ H ₁₆ O 128.22 | Insoluble Miscible | 175-175.2 NMR 96 % | 1.431-1.442 0.835-0.845 |
| 02.099 1150 | Pent-1-en-3-ol 6) |  | 3584 11717 616-25-1 | Liquid C ₅ H ₁₀ O 86.13 | Sparsely soluble Miscible | 114 NMR 98 % | 1.419-1.427 0.831-0.837 |
| 02.104 1151 | Hex-1-en-3-ol 6) |  | 3608 10220 4798-44-1 | Liquid C ₆ H ₁₂ O 100.16 | Insoluble Miscible | 133.5-134 NMR 98 % | 1.425-1.431 0.830-0.836 |
| 02.131 | But-3-en-2-ol 6) |  | 598-32-3 | Liquid C ₄ H ₈ O 72.11 | Slightly soluble Freely soluble | 90 MS 95 % | 1.409-1.415 0.831-0.837 |
| 02.136 1153 | Dec-1-en-3-ol 6) |  | 3824 51100-54-0 | Liquid C ₁₀ H ₂₀ O 156.27 | Slightly soluble Miscible | 215 NMR MS 97 % | 1.439-1.446 0.836-0.842 |
| 02.155 1842 | 1-Hepten-3-ol 6) |  | 4129 10218 4938-52-7 | Liquid C ₇ H ₁₄ O 114.19 | Practically insoluble or insoluble Freely soluble | 155 MS 97 % | 1.431-1.437 0.834-0.837 |
| 02.187 | Non-1-en-3-ol 6) |  | 10291 21964-44-3 | Liquid C ₉ H ₁₈ O 142.24 | Practically insoluble or insoluble Freely soluble | 195 MS 98 % | 1.438-1.444 0.835-0.845 |

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

| FL-no JECFA- no | EU Register name | Structural formula | FEMA no CoE no CAS no | Phys.form Mol.formul a Mol.weight | Solubility 1) Solubility in ethanol 2) | Boiling point, °C 3) Melting point, °C ID test Assay minimum | Refrac. Index 4) Spec.gravity 5) |
|-----------------------|------------------------------|---|-----------------------------|--|---|--|--|
| 07.081 1148 | Oct-1-en-3-one |  | 3515 2312 4312-99-6 | Liquid C ₈ H ₁₄ O 126.20 | Insoluble Miscible | 37-38 (3 hPa) NMR 96 % | 1.428-1.439 0.813-0.819 |
| 07.102 1147 | Pent-1-en-3-one |  | 3382 11179 1629-58-9 | Liquid C ₅ H ₈ O 84.12 | Insoluble Miscible | 68-70 (260 hPa) NMR 97 % | 1.417-1.422 0.842-0.848 |
| 07.161 | Hex-1-en-3-one |  | 1629-60-3 | Liquid C ₆ H ₁₀ O 98.14 | Practically insoluble or insoluble Freely soluble | 128 MS 95 % | 1.420-1.426 0.849-0.855 |
| 07.210 | 1-Nonene-3-one |  | 24415-26-7 | Liquid C ₉ H ₁₆ O 140.22 | Insoluble Freely soluble | 80 (16 hPa) MS 95 % | 1.436-1.442 0.826-0.830 |
| 09.281 1836 | Oct-1-en-3-yl acetate 6) |  | 3582 11716 2442-10-6 | Liquid C ₁₀ H ₁₈ O ₂ 170.25 | Practically insoluble or insoluble Freely soluble | 80 (2 hPa) NMR 97 % | 1.418-1.428 0.865-0.886 |
| 09.282 1837 | Oct-1-en-3-yl butyrate 6) |  | 3612 16491-54-6 | Liquid C ₁₂ H ₂₂ O ₂ 198.32 | Practically insoluble or insoluble Freely soluble | 81 (0.46 hPa) IR NMR MS 95 % | 1.418-1.428 0.865-0.875 |

- 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.
- 6) Stereoisomeric composition not specified.

Current Safety Evaluation Status Applying the Procedure (Based on the MSDI Approach) (JECFA, 2002c; JECFA, 2009c)

Table 2: Summary of Safety Evaluation of the JECFA substances in the present group

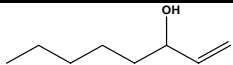
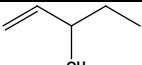
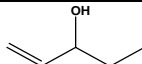
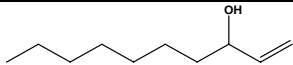
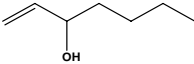
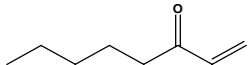
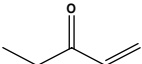
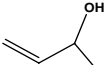
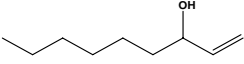
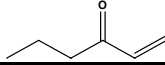
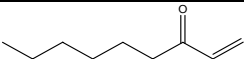
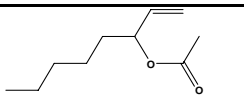
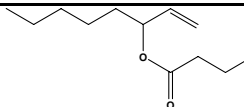
| FL-no JECFA- no | EU Register name | Structural formula | EU MSDI 1) US MSDI ($\mu\text{g/capita/day}$) | Class 2) Evaluation procedure path 3) | JECFA Outcome on the named compound [4) or 5)] | EFSA conclusion on the named compound (genotoxicity) |
|-----------------------|------------------|---|---|---|--|--|
| 02.023 1152 | Oct-1-en-3-ol |  | 240 23 | Class II A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 02.099 1150 | Pent-1-en-3-ol |  | 2.1 1 | Class II A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 02.104 1151 | Hex-1-en-3-ol |  | 0.55 2 | Class II A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 02.136 1153 | Dec-1-en-3-ol |  | ND 0.1 | Class II A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 02.155 1842 | 1-Hepten-3-ol |  | 0.13 | Class II A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 07.081 1148 | Oct-1-en-3-one |  | 1.2 0.1 | Class II A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 07.102 1147 | Pent-1-en-3-one |  | 0.29 0.1 | Class II A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 02.131 | But-3-en-2-ol |  | 0.0012 | Class II No evaluation | Not evaluated by the JECFA. | Evaluated in FGE.205, additional genotoxicity data required. |
| 02.187 | Non-1-en-3-ol |  | 0.58 | Class II No evaluation | Not evaluated by the JECFA. | Evaluated in FGE.205, additional genotoxicity data required. |
| 07.161 | Hex-1-en-3-one |  | 0.012 | Class II No evaluation | Not evaluated by the JECFA. | Evaluated in FGE.205, additional genotoxicity |

Table 2: Summary of Safety Evaluation of the JECFA substances in the present group

| FL-no JECFA- no | EU Register name | Structural formula | EU MSDI 1) US MSDI ($\mu\text{g}/\text{capita}/\text{day}$) | Class 2) Evaluation procedure path 3) | JECFA Outcome on the named compound [4) or 5)] | EFSA conclusion on the named compound (genotoxicity) |
|-----------------------|---------------------------|---|---|---|--|--|
| 07.210 | 1-Nonene-3-one |  | 0.0012 | Class II No evaluation | Not evaluated by the JECFA. | data required. Evaluated in FGE.205, additional genotoxicity data required. |
| 09.281 1836 | Oct-1-en-3-yl acetate |  | 2.1 | Class I A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |
| 09.282 1837 | Oct-1-en-3-yl butyrate |  | 0.0012 | Class I A3: Intake below threshold | 4) | Evaluated in FGE.205, additional genotoxicity data required. |

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = $\mu\text{g}/\text{capita}/\text{day}$.

2) Thresholds of concern: Class I = 1800 $\mu\text{g}/\text{person}/\text{day}$, Class II = 540 $\mu\text{g}/\text{person}/\text{day}$, Class III = 90 $\mu\text{g}/\text{person}/\text{day}$.

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.

ND Not determined.

GENOTOXICITY (*IN VITRO*)

Table 3: Summary of Additionally submitted genotoxicity data on the representative substance of subgroup 1.2.2

| FL-no | Chemical Name | Test System <i>in vitro</i> | Test Object | Concentrations tested of Substance and Test Conditions | Result | Reference | Comments |
|----------|-----------------|-----------------------------|------------------------------------|--|-----------|--------------------------|---|
| [07.102] | Pent-1-en-3-one | Reverse Mutation | <i>S. typhimurium</i> TA100 | 0 - 168.33 µg/plate* [1,4] | Positive | (Deininger et al., 1990) | Highly toxic especially without S9. Mutagenicity observed with and without S9. Reduced mutagenicity with inhibition of monooxygenase, enhanced mutagenicity with addition of epoxide hydrolase. |
| | | | | Not reported | Positive | (Eder et al., 1993) | Assay conditions, doses and control revertants were not reported but same data as in Deininger et al, 1990. |
| | | SOS chromotest | <i>E.coli</i> PQ37 | 0 - 5.05 µg* | Positive | (Deininger et al., 1990) | Highly toxic. Positive in the presence of S9-mix (induction factor of 1.83). |
| | | | | 0 - 8.41 µg* | Positive | (Eder et al., 1991a) | Reported in graphical form for MVK only, but patterns were noted to be similar for EVK, previously published by Deininger (1990). |
| | | | | 0 - 8.41 µg* | Positive | (Eder et al., 1993) | |
| | | DNA adducts | <i>E.coli</i> PQ37 | 168 mg | Positive | (Eder et al., 1993) | 1,N ² -cyclic deoxyguanosine adducts and 7-linear guanine adducts were isolated. |
| | | <i>hprt</i> assay | Mouse lymphoma L5178Y cells | 0.1 - 1.2 µg/ml [3,9] 0.5 - 5 µg/ml [5,9] 0.1 - 1.2 µg/ml [3,10] 2 - 7 µg/ml [5,9] 0.25 - 1.1 µg/ml [3,10] | Equivocal | (Lloyd, 2011a) | Highly toxic. Could only be tested in a narrow concentration range. Some indication of genotoxic effect at toxic concentrations, which was not reproducible. |
| | | Micronucleus induction | Human peripheral blood lymphocytes | 3.50 - 4.75 µg/ml [3,6], 8 - 16 µg/ml [5,6], 3 - 4 µg/ml [3,7] | Negative | (Lloyd, 2010b) | Highly toxic. Could only be tested in a narrow concentration range. Some indication of genotoxic effect at toxic concentrations, which was not reproducible. |
| [07.081] | Oct-1-en-3-one | Reverse Mutation | <i>S. typhimurium</i> TA98, TA100, | 0.32 - 1000 µg/plate [1] | Positive | (Beevers, 2009c) | All strains were negative, except TA100, with S9-mix treatment. 200 µg/plate |

Table 3: Summary of Additionally submitted genotoxicity data on the representative substance of subgroup 1.2.2

| FL-no | Chemical Name | Test System <i>in vitro</i> | Test Object | Concentrations tested of Substance and Test Conditions | Result | Reference | Comments |
|-------|---------------|-----------------------------|---|--|----------|----------------|---|
| | | | TA1535, TA1537 and TA102 | | | | resulted in 4.6-fold increase in revertants. Toxicity was observed at 1000 µg/plate. |
| | | | <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and TA102 | 15.6 - 500 µg/plate [2,3,8] | Negative | | All strains were negative, except that TA100 showed a slight 1.4-fold increase in revertants at the highest nontoxic concentration of 125 µg/plate without S9-mix treatment and a 1.2-fold increase with S9-mix at 31.25 µg/plate. Both effects were significant but not biologically relevant (2-fold over control levels). All results from pre-incubation cohort were negative. |
| | | | <i>S. typhimurium</i> TA100 | 50 - 500 µg/plate [2,5] | Negative | | |
| | | | <i>S. typhimurium</i> TA100 | 100 - 300 µg/plate [1,2], 15 - 120 µg/plate [5,8] | Positive | | 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 pre-incubation treatment that resulted in increased revertants was 45 µg/plate treatments in the presence of S9-mix (1.7-fold). |
| | | <i>hprt</i> assay | Mouse lymphoma L5178Y cells | 0.05 - 2.5 µg/ml [3,9], 0.5 - 7.5 µg/ml [5,9] 0.5 - 3 µg/ml [3,9] 0.5 - 4.5 µg/ml [5,9] 0.1 - 2.5 µg/ml [3,10] | Negative | (Lloyd, 2011c) | Highly toxic. Could only be tested in a narrow concentration range. Some indication of genotoxic effect at toxic concentrations, which was not reproducible. |
| | | Micronucleus induction | Human peripheral blood lymphocytes | 2 - 20 µg/ml [3,6], 5 - 40 µg/ml [5,6] 2 - 20 µg/ml [3,10] | Negative | (Lloyd, 2011b) | Highly toxic. Could only be tested in a narrow concentration range. Some indication of genotoxic effect at toxic concentrations, which was not reproducible. |

* Values were converted from reported µM or nM concentrations to µg values.

[1] With and without S9 metabolic activation.

[2] Plate incorporation method.

- [3] Without S9 metabolic activation.
- [4] Pre-incubation method.
- [5] With S9 metabolic activation.
- [6] 3-hour incubation with 21-hour recovery period.
- [7] 24-hour incubation with no recovery period.
- [8] Pre-incubation method with S9 metabolic activation.
- [9] 3-hour treatment.
- [10] 24-hour treatment.

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ABBREVIATIONS

| | |
|-------------|---|
| CAS | Chemical Abstract Service |
| CEF | Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CoE | Council of Europe |
| DMSO | Dimethyl Sulphoxide |
| DNA | Deoxyribonucleic acid |
| EFSA | The European Food Safety Authority |
| EU | European Union |
| EVK | Ethyl Vinyl Ketone |
| FAO | Food and Agriculture Organization of the United Nations |
| FGE | Flavouring Group Evaluation |
| FLAVIS (FL) | Flavour Information System (database) |
| GLP | Good Laboratory Practice |
| HPRT | Hypoxanthine-guanine phosphoribosyl transferase |
| ID | Identity |
| IR | Infrared spectroscopy |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| MVK | Methyl Vinyl Ketone |
| MF | Mutant frequency |
| MNBN | MicroNucleated BiNucleate cells |
| MS | Mass spectra |
| MSDI | Maximised Survey-derived Daily Intake |
| mTAMDI | Modified Theoretical Added Maximum Daily Intake |
| NMR | Nuclear Magnetic Resonance |
| No | Number |
| NOAEL | No observed adverse effect level |
| NTP | National Toxicology Program |
| OECD | Organisation for Economic Co-operation and Development |

| | |
|--------|---|
| PHA | Phytohaemagglutinin |
| (Q)SAR | (Quantitative) Structure Activity Relationship |
| RI | Replication index |
| RS | Relative survival |
| SCF | Scientific Committee on Food |
| SKF | 2'-Diethylaminoethyl 2,2-diphenylpentanoate hydrochloride |
| SOSIP | SOS inducing potency |
| WHO | World Health Organisation |