EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 226 (FGE.226): Consideration of genotoxicity data on one \(-\)-unsaturated aldehyde from chemical subgroup 1.1.1(b) of FGE.19 by EFSA

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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 evaluate the genotoxic potential of one flavouring substance from subgroup 1.1.1(b) of FGE.19 in the Flavouring Group Evaluation 226. The Flavour Industry has provided additional genotoxicity studies for the substance [FL-no: 16.071] in FGE.226. Based on these new data the Panel concluded that 4,5-epoxydec-2(trans)-enal did not induce gene mutations in bacterial cells but was positive in an in vitro micronucleus assay, so, 4,5-epoxydec-2(trans)-enal is considered an in vitro genotoxic agent. The negative results obtained in an in vivo micronucleus assay cannot overrule the positive results of the in vitro micronucleus assay with and without S9-mix due to the lack of cytotoxicity in the bone marrow. On this basis, an in vivo Comet assay in rodents is recommended in order to verify possible genotoxic effects at the first site of contact (e.g., stomach/duodenum cells).

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KEY WORDS

4,5-Epoxydec-2(trans)-enal, Subgroup 1.1.1, FGE.19, FGE.200, FGE.226.

1 On request from the Commission, Question No EFSA-Q-2012-00077, adopted on 5 July 2012.
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SUMMARY

The European Food Safety Authority (EFSA) asked the Scientific 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 requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The present Flavouring Group Evaluation 226 (FGE.226), corresponding to subgroup 1.1.1(b) of FGE.19, concerns one α,β-unsaturated aldehyde which also include an epoxide, 4,5-epoxydec-2(trans)-enal [FL-no: 16.071]. The α,β-unsaturated aldehyde structure is considered to be a structural alert for genotoxicity and the data on genotoxicity previously available did not rule out the concern for genotoxicity.

To evaluate the genotoxic potential of 4,5-epoxydec-2(trans)-enal [FL-no: 16.071] the Panel has therefore requested additional genotoxicity data according to the test strategy worked out by the Panel.

According to the above requirements, the Industry has submitted additional genotoxicity studies for 4,5-epoxydec-2(trans)-enal.

Based on these new data the Panel noted that 4,5-epoxydec-2(trans)-enal did not induce gene mutations in bacterial cells but was positive in an in vitro micronucleus assay, so, 4,5-epoxydec-2(trans)-enal is considered an in vitro genotoxic agent. The negative results obtained in an in vivo micronucleus assay cannot overrule the positive results of the in vitro micronucleus assay with and without S9-mix due to the lack of cytotoxicity in the bone marrow. On this basis, an in vivo Comet assay in rodents is recommended in order to verify possible genotoxic effects at the first site of contact (e.g., stomach/duodenum cells).
TABLE OF CONTENTS

Abstract .................................................................................................................................................... 1
Summary .................................................................................................................................................. 2
Background ............................................................................................................................................. 4
Terms of reference .................................................................................................................................. 5
History of Evaluation ............................................................................................................................... 5
Assessment ............................................................................................................................................... 5
1. Presentation of the substance in FGE.226 .......................................................................................... 5
   1.1. Description ................................................................................................................................... 5
   1.2. Subgroup 1.1.1(b) ................................................................................................................... 6
2. Additionally submitted genotoxicity data on 4,5-epoxydec-2(trans)-enal of subgroup 1.1.1(b) ....... 6
   2.1. In vitro Data .................................................................................................................................. 6
       2.1.1. Bacterial Reverse Mutation Assay ...................................................................................... 6
       2.1.2. Micronucleus Assays .......................................................................................................... 7
   2.2. In vivo Data .................................................................................................................................... 8
       2.2.1. In vivo Micronucleus Assays ............................................................................................. 8
   2.3. Discussion of Mutagenicity/Genotoxicity Data ...................................................................... 9
3. Conclusion ....................................................................................................................................... 10
Specification Summary of the Substance in the FGE.200Rev2 (JECFA, 2009b) .................................. 11
Current Safety Evaluation Status Applying the Procedure (Based on Intakes Calculated by the MSDI
 Approach) (JECFA, 2002c) .................................................................................................................... 11
Genotoxicity (in vitro) .......................................................................................................................... 12
Genotoxicity (in vivo) ............................................................................................................................ 13
References ............................................................................................................................................. 14
Abbreviations ......................................................................................................................................... 16
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 28 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 alpha,beta-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 some of these data requested on genotoxicity.

**TERMS OF REFERENCE**

The European Commission requests the European Food Safety Authority to carry out a safety assessment on 4,5-epoxydec-2(trans)-enal [FL-no: 16.071], in accordance with Commission Regulation (EC) No 1565/2000.

**HISTORY OF EVALUATION**

The present Flavouring Group Evaluation 226 (FGE.226) concerns the evaluation of the genotoxic properties of one aliphatic aldehyde with the $\alpha$,$\beta$-unsaturation in conjugation with an epoxide moiety. This substance was originally allocated to FGE.200 (FGE.19 subgroup 1.1.1).

Subgroup 1.1.1 of FGE.19 originally covers 71 $\alpha$,$\beta$-unsaturated aliphatic aldehydes. Seventy of these are simple, aliphatic, $\alpha$,$\beta$-unsaturated aldehydes, or precursors for such, with or without additional double bonds, which is not in conjugation with the $\alpha$,$\beta$-unsaturated structure. These 70 substances were allocated to subgroup 1.1.1(a) in FGE.200. The one remaining aliphatic, $\alpha$,$\beta$-unsaturated aldehyde contains an epoxide moiety which is not present within the other 70 members of FGE.19 subgroup 1.1.1. On this basis, it would be anticipated to have different chemical reactivity potential, and would have metabolic options that are not available to the other members of this subgroup. For these reasons, the Panel decided that this substance should be allocated to a separate subgroup, subgroup 1.1.1(b) and evaluated in a separate FGE, FGE.226.

The present FGE.226 deals with the evaluation of the genotoxicity data submitted by the Flavour Industry for substance 4,5-epoxydec-2(trans)-enal [FL-no: 16.071] from subgroup 1.1.1(b).

**ASSESSMENT**

1. **PRESENTATION OF THE SUBSTANCE IN FGE.226**

1.1. **Description**

The present Flavouring Group Evaluation 226 (FGE.226), corresponding to subgroup 1.1.1(b) of FGE.19, concerns one aliphatic aldehyde with the $\alpha$,$\beta$-unsaturation in conjugation with a epoxide moiety, 4,5-epoxydec-2(trans)-enal [FL-no: 16.071]. The substance is shown in Table 1.

The substance has previously been evaluated by the JECFA (JECFA, 2009c). A summary of the current evaluation status by the JECFA and the outcome of the present consideration is presented in Table 2.
1.2. Subgroup 1.1.1(b)

As the α,β-unsaturated aldehyde and ketone structures are considered alerts for genotoxicity (EFSA, 2008b) and the data on genotoxicity previously available did not rule out the concern for genotoxicity, the Panel has requested additional genotoxicity data for 4,5-epoxydec-2(trans)-enal according to the test strategy (EFSA, 2008bb). The chemical structure of the substance is shown in Table 1.1

<table>
<thead>
<tr>
<th>FL-no</th>
<th>Subgroup</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
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<td>4,5-Epoxydec-2(trans)-enal</td>
<td><img src="attachment.png" alt="Structural formula" /></td>
<td>-</td>
<td>188590-62-7</td>
</tr>
</tbody>
</table>

2. ADDITIONALLY SUBMITTED GENOTOXICITY DATA ON 4,5-EPOXYDEC-2(TRANS)-ENAL OF SUBGROUP 1.1.1(B)

Introduction

The Industry has submitted in vitro and in vivo genotoxicity data for the representative and only substance for this subgroup 1.1.1(b), 4,5-epoxydec-2(trans)-enal [FL-no: 16.071] (EFFA, 2011n).

2.1. In vitro Data

In vitro genotoxicity assays have been performed in bacteria and mammalian cells with the α,β-unsaturated aldehyde 4,5-epoxydec-2(trans)-enal [FL-no: 16.071].

2.1.1. Bacterial Reverse Mutation Assay

An Ames assay was conducted in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 to assess the mutagenicity of 4,5-epoxydec-2(trans)-enal, both in the absence and in the presence of metabolic activation by (S9-mix), in two experiments (Sokolowski, 2001b). It is a GLP study conducted in accordance with OECD Guideline 471. An initial toxicity range-finding experiment was carried out in the absence and presence of S9-mix in strains TA98 and TA100 only, using final concentrations of 4,5-epoxydec-2(trans)-enal at 3, 10, 33, 100, 333 and 1000 μg/plate, plus negative (solvent) and positive controls. Evidence of toxicity, in terms of a decrease in revertant count, was apparent on all plates treated at 333 μg/plate and above in the absence and at 1000 μg/plate in the presence of S9-mix. The first experiment then evaluated all five strains in the absence and presence of S9-mix using plate incorporation methodology and final concentrations of either 1, 3, 10, 33, 100, and 333 μg/plate (TA1535, TA1537 and TA102 without S9-mix) or 3, 10, 33, 100, 333 and 1000 μg/plate (TA1535, TA1537 and TA102 with S9-mix; TA100 and TA98 with and without S9-mix). Following these treatments, evidence of toxicity was observed in all strains at concentrations of 333 and/or 1000 μg/plate, both in the absence and in the presence of S9-mix. No strains produced a biologically significant increase in the number of revertants.

In the second experiment, treatments of all the tester strains were performed in the absence and presence of S9-mix using the same concentrations as in the first experiment. In order to increase the range of mutagenic detection, a second experiment was performed using the pre-incubation methodology in the absence and presence of S9-mix using the same concentrations as in the first experiment. Following these treatments, evidence of toxicity was again observed in all strains at
concentrations of 333 and/or 1000 µg/plate. No biologically significant increases in the number of revertants were seen in any strain.

It was concluded that 4,5-epoxydec-2(trans)-enal did not induce mutations in five strains of *S. typhimurium* when tested up to toxic concentrations in the absence and in the presence of a rat liver metabolic activation system (Sokolowski, 2001b).

### 2.1.2. Micronucleus Assays

4,5-Epoxydec-2(trans)-enal was assayed for the induction of chromosome damage, and potential aneugenic effects, in mammalian cells *in vitro* by examining the effect on the frequency of micronuclei in cultured human peripheral blood lymphocytes (whole blood cultures pooled from two healthy volunteers) treated in the absence and presence of rat liver metabolizing system (S9-mix) (Lloyd, 2009c). This GLP study complies with OECD Guideline 487. 4,5-Epoxydec-2(trans)-enal was added at 48 hours following culture initiation (stimulation by phytohaemagglutinin) either for 3 hours in the absence or presence of S9-mix, or for 24 hours in the absence of S9-mix. Cytochalasin B (6 µg/ml) was added either at the start of treatment (24-hour treatments) or at the start of recovery (after 3-hour treatments) in order to block cytokinesis and generate binucleate cells for analysis. It remained in the cultures until they were harvested 24 hours after the start of treatment. A preliminary range-finding experiment had been conducted with and without S9-mix treatment in order to determine the effect of treatment upon Replication Index (RI), which was used as a basis for choosing a range of concentrations to be evaluated in the main study.

In the main assay, micronuclei were analysed at multiple concentrations for each treatment group. For 3-hour treatment without S9-mix the concentrations were 1.0, 2.0, 4.0 and 5.0 µg/ml, for 3-hour treatment with S9-mix the concentrations were 9.0, 10.5, and 12.0 µg/ml, and for 24-hour treatment without S9-mix the concentrations were 2.5, 3.0, 3.5 and 4.0 µg/ml. The levels of cytotoxicity (reduction in RI) at the top concentrations reached 61, 52 and 55 % in the 3-hour treatment in the absence of S9-mix, the 3-hour treatment in the presence of S9 and the 24-hour treatment in the absence of S9, respectively. These are within or very close to the target (50 - 60 %) range. One thousand binucleate cells per culture from two replicate cultures per concentration were scored for micronuclei. The study is therefore considered to comply with OECD Guideline 487.

Following the 3-hour treatment without S9-mix, there was an increase in the frequency of micronucleated binucleate cells (MNBN) from 0.1 % in the solvent control to 0.65 % (p ≤ 0.01) and 0.45 % (p ≤ 0.05) at the two highest concentrations. However, the increases observed at 4.000 and 5.000 µg/mL were small and were exaggerated because the MNBN cell frequencies in both vehicle control cultures (0.1% in both cases) were at the lower end of the normal range (0 to 1.0 %). Furthermore, the MNBN cell frequencies in all treated cultures under this treatment condition fell within the 95th percentile of the normal range. Therefore these observations were not considered by the Authors to represent clear evidence of a biologically relevant response, although the results cannot be considered clearly negative.

Following the 3-hour treatment in the presence of S9-mix at the highest concentration analysed (12.0 µg/mL), the frequency of MNBN cells (2.25 %) was significantly higher (p ≤ 0.001) than those observed in concurrent vehicle controls (0.2 %). The MNBN cell frequencies in both cultures at 12.00 µg/mL exceeded the normal ranges, and therefore this was considered to be a positive result. Similarly, for the 24-hour treatment at the lowest (2.5 µg/mL) and two highest concentrations (3.5 and 4.0 µg/mL), the frequencies of MNBN cells were significantly higher (1.25 % p ≤ 0.05, 3.19 % p ≤ 0.001 and 3.80 % p ≤ 0.001, respectively) than those observed in the concurrent vehicle control (0.65 %). The MNBN cell frequencies in both cultures at each of these concentrations exceeded the normal ranges, and therefore this was again considered to be a positive result (Lloyd, 2009c).

On the basis of these results, a new GLP study to determine whether 4,5-epoxydec-2(trans)-enal was acting as a clastogen or an aneugen using fluorescence *in situ* hybridization (FISH) analysis was
attempted (Lloyd, 2011e). Micronuclei were analysed at multiple concentrations for each treatment group, and the maximum concentrations were based on the toxicity displayed in the previous study. For 3-hour treatment with S9-mix the concentrations were 0, 12.0, 15.0 and 17.5 \( \mu \)g/ml, with MNBN cell frequencies of 0.30 %, 0.20 %, 0.50 % and 0.45 % respectively, with a historical control range of 0.0 - 0.7 %. For 24-hour treatment without S9-mix the concentrations were 0, 4.0, 5.0 and 7.5 \( \mu \)g/ml, with MNBN cell frequencies of 0.35 %, 0.25 %, 0.55 % and 0.20 % respectively, with a historical control range of 0.1 - 0.9 %. The levels of cytotoxicity (reduction in replication index, RI) were 16, 36 and 48 % for the three concentrations in the 3-hour treatment in the presence of S9-mix and 3, 10 and 56 % for the three concentrations in the 24-hour treatment in the absence of S9-mix, respectively. 48 and 56 % at the top concentrations are within or very close to the target (50 - 60 %) range. One thousand binucleate cells per culture from 2 replicate cultures per concentration were scored for micronuclei. The study is therefore considered to comply with OECD Guideline 487.

The MNBN cell frequencies in all cultures under both treatment conditions fell within the normal range, thereby giving clear negative results. These data are in marked contrast to the previously described study (Lloyd, 2009c). Because no induction of micronuclei was observed following 3+21 hours with S9-mix and 24+0 hours without S9-mix treatments, further analysis (FISH) was not conducted. Different blood donors were used in the first and second studies on 4,5-epoxydec-2(trans)-enal. A subsequent study in which peripheral blood from the donors used in both experiments were compared in a single experiment confirmed the existence of a donor effect for this compound (data not provided). It is not known why this difference occurred, but the positive responses observed in the previous study (Lloyd, 2009c) cannot be dismissed.

2.2. **In vivo Data**

2.2.1. **In vivo Micronucleus Assays**

On the basis of the *in vitro* micronucleus studies reported above, it was concluded that 4,5-epoxydec-2(trans)-enal was an *in vitro* clastogen for human lymphocytes, although a donor effect, as described above, confounded the interpretation of the results. It was decided that it was most appropriate to carry out an *in vivo* micronucleus assay to determine whether the results obtained in the initial *in vitro* micronucleus assay could be confirmed *in vivo*. Therefore, groups of Han-Wistar rats were administered 4,5-epoxydec-2(trans)-enal via gavage and the induction of micronuclei in the polychromatic erythrocyte (PCE) of the bone marrow of treated rats was examined (Henderson D, 2011).

In an initial range-finding experiment to identify a maximum tolerated dose (MTD), groups of male and female (up to 3 animals/sex/group) Han-Wistar rats were administered 4,5-epoxydec-2(trans)-enal by oral gavage at doses of 250, 350, 500, 700, 1000, 1400, and 2000 mg/kg bw/day until an estimate of the MTD was established. Animals were dosed once daily for two consecutive days with the test article and observations made over a 2-day period following the final administration. Clinical signs of toxicity and body weight were recorded. At doses of 500 mg/kg bw/day and above, clinical signs of toxicity such as decreased activity and pilo-erection were observed in all animals, and mortality was induced. At doses of 350 mg/kg bw/day and below no clinical signs of toxicity were observed, except in one female at 350 mg/kg bw/day, for which decreased activity, pilo-erection and hunched posture were observed. Both male and female groups at 350 mg/kg bw/day showed mean body weight loss. On the basis of these concentrations, the MTD was considered to be 350 mg/kg bw/day. Additionally, as there were no substantive differences between sexes in apparent toxicity, only male animals were subsequently used in the micronucleus experiment.

In the micronucleus experiment, groups of male (6 animals/group) rats were administered 4,5-epoxydec-2(trans)-enal by oral gavage at 87.5, 175 and 350 mg/kg bw/day on 2 occasions 24 hours apart. Animals were sampled 24 hours after the final administration, thus enabling examination of cells exposed to the test article over a period of 24 to 48 hours prior to sampling. At the highest dose
on day 2, decreased activity was observed in all animals 1-hour post dose and at 2-hour post dose,
pilo-erection was also noted in all animals. For the highest dose group one animal was found dead at
end of day 2. These observations provide some indications that the animals were systemically exposed
to the 4,5-epoxydec-2(trans)-enal.

Rats treated with 4,5-epoxydec-2(trans)-enal at all doses exhibited group mean % PCE that were
similar to the vehicle control group. These values were comparable with the historical control data for
this experiment at the testing laboratory, thus confirming there was no evidence of test article related
bone marrow toxicity. Additionally, rats treated with 4,5-epoxydec-2(trans)-enal at all doses exhibited
MN PCE frequencies that were similar to the vehicle control group and which were considered
consistent with the laboratory's historical data. There were no statistically significant increases in
micronucleus frequency for any of the groups receiving the test article, compared to the concurrent
vehicle control. On this basis, it was concluded that 4,5-epoxydec-2(trans)-enal did not induce
micronuclei in the polychromatic erythrocytes of the bone marrow of male rats treated up to 350
mg/kg/day (a dose which exceeded the maximum tolerated dose).

2.3. Discussion of Mutagenicity/Genotoxicity Data

4,5-Epoxydec-2(trans)-enal was unable to induce gene mutations in a valid Ames test. In a valid
in vitro micronucleus assay, 4,5-epoxydec-2(trans)-enal was clearly positive in both treatments for 3+21
hours in the presence of S9-mix and for 24+0 hours in the absence of S9-mix. In the same study, in the
treatment for 3+21 hours in the absence of S9-mix, statistically significant increases of MNBN cell
frequencies were reported at the two highest concentrations. These increases were not considered
biologically relevant because the MNBN cell frequencies in the vehicle control cultures (0.1 %) were
at the lower end of the historical control range (0.0 to 1.0 %) and because all the MNBN cell
frequencies fell within the 95th percentile of the normal range. On this basis, the results of this study
should be considered as equivocal. Overall, the results of this study indicate that 4,5-epoxydec-
2(trans)-enal is an in vitro genotoxic agent both in the presence and in the absence of metabolic
activation.

The positive results of the first study (Lloyd, 2009c) could not be confirmed in a second study, in
which different blood donors were used (Lloyd, 2011e). According to the Authors, the existence of a
donor effect for this substance was confirmed in a subsequent study in which peripheral blood from
the donors used in both studies were compared in a single experiment. However, data related to this
experiment were not provided and also an explanation for this difference was not given. Therefore, the
concern for the genotoxic potential of 4,5-epoxydec-2(trans)-enal remains.

4,5-Epoxydec-2(trans)-enal was found negative in a valid in vivo micronucleus assay in rats treated by
oral gavage up to 350 mg/kg bw, considered as the MTD, on two occasions 24 hours apart. At this
dose and below, no clinical signs of toxicity were observed, except one female; both male and female
groups showed only mean body weight loss. Clinical signs, including some mortality, were observed
at the dose of 500 mg/kg bw, used in the initial range-finding experiment. At 350 mg/kg bw, there was
no evidence of any test article-induced toxicity to the bone marrow. The Panel considered that
lethality may indicate that the bone marrow was exposed, however this is not a proof. In addition, the
negative results of this in vivo micronucleus assay do not allow to exclude site of contact effects.
Therefore, an in vivo Comet assay should be performed.

The request for a Comet assay is in line with the recommendations of the AFC Panel (EFSA, 2008bb)
and Scientific Committee opinion on genotoxicity testing strategies applicable to food and feed safety
assessment (EFSA, 2011ae).
3. CONCLUSION

4,5-Epoxydec-2(trans)-enal did not induce gene mutations in bacterial cells (Ames test). It was positive in an in vitro micronucleus assay in cultured human lymphocytes with and without metabolic activation. Although these results could not be confirmed in a second study in which different blood donors were used, 4,5-epoxydec-2(trans)-enal is considered an in vitro genotoxic agent in the presence and in the absence of S9-mix. The negative results obtained in an in vivo micronucleus assay do not allow to exclude possible first site of contact effects. In addition, the Panel considered that lethality may indicate that the bone marrow were exposed, however this is not a proof. On this basis, an in vivo Comet assay in rodents is required, in order to verify possible genotoxic effects at the first site of contact (e.g., stomach/duodenum cells).

The request for a Comet assay is in line with the recommendations of the AFC Panel (EFSA, 2008bb) and Scientific Committee opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA, 2011ae).
### Table 1: Specification Summary of the Substances in the Present Group (JECFA, 2009b)

<table>
<thead>
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<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Mol.formula</th>
<th>Mol.weight</th>
<th>Solubility 1)</th>
<th>Solubility in ethanol 2)</th>
<th>Boiling point, °C 3)</th>
<th>Melting point, °C</th>
<th>ID test</th>
<th>Assay minimum</th>
<th>Refrac. Index 4)</th>
<th>Spec.gravity 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.071</td>
<td>1570</td>
<td>4,5-Epoxydec-2(trans)-enal</td>
<td><img src="image" alt="Structural formula" /></td>
<td>-</td>
<td>-</td>
<td>188590-62-7</td>
<td>Liquid</td>
<td>C₁₀H₁₆O₂</td>
<td>168.23</td>
<td>Soluble</td>
<td>Soluble</td>
<td>80-83 (0.8 hPa)</td>
<td>IR NMR MS</td>
<td>87 %</td>
<td></td>
<td></td>
<td>1.472-1.478</td>
</tr>
</tbody>
</table>

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.

### Current Safety Evaluation Status Applying the Procedure (Based on Intakes Calculated by the MSDI Approach) (JECFA, 2002c)

### Table 2: Summary of Safety Evaluation of the JECFA Substance in the Present Group (JECFA, 2009c)

<table>
<thead>
<tr>
<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>EU MSDI 1)</th>
<th>US MSDI (µg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>JECFA Outcome on the named compound [4) or 5])</th>
<th>EFSA conclusion on the named compound (genotoxicity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.071</td>
<td>1570</td>
<td>4,5-Epoxydec-2(trans)-enal</td>
<td><img src="image" alt="Structural formula" /></td>
<td>0.061</td>
<td>0.2</td>
<td>Class III A3: Intake below threshold</td>
<td>4) Evaluated in FGE.200, additional genotoxicity data required. Evaluated in FGE.226, an in vivo Comet assay is requested.</td>
<td></td>
</tr>
</tbody>
</table>

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

**Table 3:** Summary of Additionally Submitted *In Vitro* Genotoxicity Data on 4,5-Epoxydec-2(trans)-enal

<table>
<thead>
<tr>
<th>FL-no</th>
<th>Chemical Name</th>
<th>Test System <em>in vitro</em></th>
<th>Test Object</th>
<th>Concentrations of Substance and Test Conditions</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>[16.071]</td>
<td>4,5-Epoxydec-2(trans)-enal</td>
<td>Reverse Mutation</td>
<td><em>S. typhimurium</em> TA98, TA100</td>
<td>3-1000 μg/plate [1,2] 3-1000 μg/plate [1,3]</td>
<td>Negative</td>
<td>(Sokolowski, 2001b)</td>
<td>Valid GLP study in compliance with OECD Guideline 471</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Micronucleus induction</td>
<td>Human peripheral blood lymphocytes</td>
<td>1.0-5.0 μg/ml [4,6]</td>
<td>Equivocal</td>
<td>(Lloyd, 2009c)</td>
<td>Valid GLP study in compliance with OECD Guideline 487. Increases at 4.0 and 5.0 micrograms/ml are of doubtful biological relevance due to low vehicle control and because are within the 95th percentile of the normal range.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.0-12.0 μg/ml [5,6], 2.5-4.0 μg/ml [4,7]</td>
<td>Positive</td>
<td></td>
<td>Complies with draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.0-17.5 μg/ml [5,6], 4.0-7.5 μg/ml [4,7]</td>
<td>Negative</td>
<td>(Lloyd, 2011e)</td>
<td>Complies with draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study.</td>
</tr>
</tbody>
</table>

* Values were converted from reported μM or nM concentrations to μg values.
  
[1] With and without S9 metabolic activation.
[6] 3-hours incubation with 21-hours recovery period.
[7] 24-hours incubation with no recovery period.
**GENOTOXICITY (IN VIVO)**

Table 4: Table 4: Summary of Additionally Submitted *In Vivo* Genotoxicity Data on 4,5-Epoxydec-2(trans)-enal

<table>
<thead>
<tr>
<th>FL-no</th>
<th>Chemical Name</th>
<th>Test System in vivo</th>
<th>Test Object Sex/No per group</th>
<th>Route</th>
<th>Concentrations of Substance and Test Conditions</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>[16.071]</td>
<td>4,5-Epoxydec-2(trans)-enal</td>
<td>Micronucleus assay</td>
<td>Han-Wistar rats Male / 6</td>
<td>Gavage</td>
<td>87.5, 175, and 350 mg/kg bw/day</td>
<td>Negative</td>
<td>(Henderson, 2011)</td>
<td>Complies with draft OECD guideline 474. No evidence of test article related bone marrow toxicity at the top dose.</td>
</tr>
</tbody>
</table>
REFERENCES


ABBREVIATIONS

CAS Chemical Abstract Service
CEF Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE Council of Europe
CPA Cyclophosphamide
EFSA The European Food Safety Authority
EU European Union
FAO Food and Agriculture Organization of the United Nations
FGE Flavouring Group Evaluation
FLAVIS (FL) Flavour Information System (database)
ID Identity
IR Infrared spectroscopy
JECFA The Joint FAO/WHO Expert Committee on Food Additives
MMC Mitomycin
MNBN Micronucleated Binucleated
MSDI Maximised Survey-derived Daily Intake
mTAMDI Modified Theoretical Added Maximum Daily Intake
MTD Maximum tolerated dose
No Number
NOAEL No observed adverse effect level
NTP National Toxicology Program
OECD Organisation for Economic Co-operation and Development
PCE Polychromatic erythrocyte
PHA Phytohaemagglutinin
(Q)SAR (Quantitative) Structure-Activity Relationship
RI Replication Index
SCF Scientific Committee on Food
VIN Vinblastine
WHO  World Health Organisation