EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 200 (FGE.200): 74 , -unsaturated aldehydes and precursors from subgroup 1.1.1 of FGE.19

EFSA publication; Beltoft, Vibe Meister; Binderup, Mona-Lise; Frandsen, Henrik Lauritz; Lund, Pia; Nørby, Karin Kristiane

Link to article, DOI:
10.2903/j.efsa.2014.3709

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 200 (FGE.200): 74 α,β-unsaturated aldehydes and precursors from subgroup 1.1.1 of FGE.19[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate the genotoxic potential of 74 flavouring substances from subgroup 1.1.1 of FGE.19 in the Flavouring Group Evaluation 200 (FGE.200). The Flavour Industry has provided additional genotoxicity studies for one representative substance in FGE.200, namely hex-2(*trans*)-enal [FL-no 05.073], and for other two substances in the same subgroup, namely 2-dodecenal [05.037] and 2-nonenal [05.171]. The Panel has evaluated these data and concluded that the concern still remains with respect to genotoxicity for the substances of this subgroup and their three representative substances. The Panel confirms, the need for an *in vivo* Comet assay performed in duodenum and liver for hex-2(*trans*)-enal [FL-no: 05.073]. For the two other representative substances of subgroup 1.1.1 (nona-2(*trans*),6(*cis*)-dienal [FL-no: 05.058] and oct-2-enal [FL-no: 05.060]), a combined *in vivo* Comet assay and micronucleus assay would be required. For the latter, evidence of bone marrow exposure should be provided.

KEY WORDS

α,β-unsaturated aldehydes, straight chain, FGE.200, flavouring substances, safety evaluation, subgroup 1.1.1, FGE.19

[1] On request from the European Commission, Question No EFSA-Q-2013-00445 to 00518, adopted on 21 May 2014.

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Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 200 (FGE.200): 74 α,β-unsaturated aldehydes and precursors from subgroup 1.1.1 of FGE.19. EFSA Journal 2014;12(6):3709, 50 pp. doi:10.2903/j.efsa.2014.3709

Available online: www.efsa.europa.eu/efsajournal

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 200 (FGE.200) concerns 74 substances, corresponding to subgroup 1.1.1 of FGE.19. These substances are 74 straight chain, α,β-unsaturated aldehydes, with or without additional non-conjugated double-bonds, or precursors for such structures.

Information on one representative material hex-2(*trans*)-enal [FL-no: 05.073] and two other substances, 2-nonenal [FL-no: 05.171] and 2-dodecenal [FL-no: 05.037] from subgroup 1.1.1, has now been submitted by the European Flavour Association. This information is intended to cover the re-evaluation of the above mentioned substances and of the following 71 substances from FGE.19 subgroup 1.1.1.

The new data submitted are related only to one representative substance of subgroup 1.1.1, hex-2(trans)-enal [FL-no: 05.073].

For hex-2(trans)-enal [FL-no: 05.073] gene mutations were observed *in vitro* in TA100, and chromosomal aberrations were observed *in vitro* likewise. In addition, a biomonitoring study in human buccal cells showed a statistically significant increase in the frequency of micronuclei at concentrations that might be relevant for the use of hex-2(*trans*)-enal as flavouring substance. The new submitted study performed on a Muta^{TM}Mouse model does not cover these endpoints adequately. The Panel noted that overall the available experimental data from animals and humans, while not showing an induction of gene mutations, do not allow to assess the potential clastogenic activity of hex-2(*trans*)-enal at the first site of contact and in the liver where higher levels of DNA adducts were observed than in other tissues investigated. Therefore, the new data provided by the Industry do not rule out the genotoxicity concern for the substances of subgroup 1.1.1.

For both 2-dodecenal and 2-nonenal tested through micronucleus assays in mouse bone marrow PCE (Honarvar, 2007b; Honarvar, 2008) there was no direct confirmation that the bone marrow was exposed, as no toxicokinetic measures of the test substance in plasma were made.

Under these conditions, the Panel confirms the need for an *in vivo* Comet assay performed in duodenum and liver for hex-2(*trans*)-enal [FL-no: 05.073]. For the two other representative substances of subgroup 1.1.1 (nona-2(*trans*),6(*cis*)-dienal [FL-no: 05.058] and oct-2-enal [FL-no: 05.060]), a combined *in vivo* Comet assay and micronucleus assay would be required. For the latter, evidence of bone marrow exposure should be provided.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

In the 26th Plenary meeting of the AFC Panel on 27-29 November 2007, EFSA discussed the flavouring group evaluation 19 (FGE.19). FGE.19 contains those flavouring substances which are α,β-unsaturated aldehydes or ketones and their precursors which could give rise to such carbonyl substances via hydrolysis and/or oxidation. The α,β-unsaturated aldehyde and ketone structure is considered by the Panel to be a structural alert for genotoxicity. FGE.19 was divided into subgroups. For subgroup 1.1.1 EFSA concluded that there is a need for additional information before conclusions on the substances in this subgroup can be reached. On 21 February 2011, EFSA adopted a statement on earlier data provided by the European Flavour Association concerning this subgroup. However, the conclusion was that the need for additional genotoxicity data had not been alleviated and genotoxicity studies should be carried out for the representative substances.

Information on one representative material hex-2(trans)-enal [FL-no: 05.073] and two other substances, 2-nonenal [FL-no: 05.171] and 2-dodecenal [FL-no: 05.037] from subgroup 1.1.1, has now been submitted by the European Flavour Association. This information is intended to cover the re-evaluation of the above mentioned substances and of the following 71 substances from FGE.19 subgroup 1.1.1:

- Hex-2-en-1-ol [FL-no: 02.020]
- Nona-2,6-dien-1-ol [FL-no: 02.049]
- Pent-2-en-1-ol [FL-no: 02.050]
- Non-2(trans)-en-1-ol [FL-no: 02.090]
- Non-2(cis)-en-1-ol [FL-no: 02.112]
- Dec-2-en-1-ol [FL-no: 02.137]
- Hex-2(cis)-en-1-ol [FL-no: 02.156]
- Oct-2-en-1-ol [FL-no:02.192]
- Undec-2-en-1-ol [FL-no: 02.210]
- Tr-2, cis-6-Nonadien-1-ol [FL-no: 02.231]
- 2-Dodecenal [FL-no: 05.037]
- Nona-2(trans),6(cis)-dienal [FL-no: 05.058]
- Oct-2-enal [FL-no: 05.060]
- 2-Heptenal [FL-no: 05.070]
- trans-2-Nonenal [FL-no: 05.072]
- Hex-2(trans)-enal [FL-no: 05.073]
- Dec-2-enal [FL-no: 05.076]
- Tridec-2-enal [FL-no: 05.078]

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

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

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

- Pent-2-enal [FL-no: 05.102]
- 2-Undecenal [FL-no: 05.109]
- Octa-2(trans),6(trans)-dienal [FL-no: 05.111]
- 4-Methylpent-2-enal [FL-no: 05.114]
- Dodeca-2,6-dienal [FL-no: 05.120]
- Dodec-2(trans)-enal [FL-no: 05.144]
- Hept-2(trans)-enal [FL-no: 05.150]
- 2-Nonenal [FL-no: 05.171]
- Nona-2(trans),6(trans)-dienal [FL-no: 05.172]
- Tetradec-2-enal [FL-no: 05.179]
- Undec-2(trans)-enal [FL-no: 05.184]
- 2-Hexenal [FL-no: 05.189]
- trans-2-Octenal [FL-no: 05.190]
- trans-2-Decenal [FL-no: 05.191]
- trans-2-Tridecenal [FL-no: 05.195]
- 1,1-Diethoxynona-2,6-diene [FL-no: 06.025]
- 1,1-Diethoxyhex-2-ene [FL-no: 06.031]
- 1,1-Dimethoxyhex-2(trans)-ene [FL-no: 06.072]
- Allyl butyrate [FL-no: 09.054]
- Allyl heptanoate [FL-no: 09.097]
- Allyl nonanoate [FL-no: 09.109]
- Allyl octanoate [FL-no: 09.119]
- Allyl undec-10-enoate [FL-no: 09.146]
- Allyl propionate [FL-no: 09.233]
- Allyl hexanoate [FL-no: 09.244]
- Allyl crotonate [FL-no: 09.247]
- Oct-2-enyl acetate [FL-no: 09.276]
- Oct-2(trans)-enyl butyrate [FL-no: 09.277]
- Hept-2-enyl isovalerate [FL-no: 09.303]
- Allyl hexa-2,4-dienoate [FL-no: 09.312]
- Hept-2-enyl acetate [FL-no: 09.385]
- Hex-2(trans)-enyl acetate [FL-no: 09.394]
- Hex-2(trans)-enyl propionate [FL-no: 09.395]
- Hex-2-enyl butyrate [FL-no: 09.396]
- Hex-2-enyl formate [FL-no: 09.397]
- Hex-2-enyl hexanoate [FL-no: 09.398]
- Hex-2-enyl isovalerate [FL-no: 09.399]
- Hex-2-enyl phenylacetate [FL-no: 09.400]
- Allyl 2-ethylbutyrate [FL-no: 09.410]
- Allyl cyclohexanebutyrate [FL-no: 09.411]
- Allyl cyclohexanevalerate [FL-no: 09.469]
- Allyl cyclohexaneacetate [FL-no: 09.482]
- Allyl isovalerate [FL-no: 09.489]
- Allyl cyclohexanehexanoate [FL-no: 09.492]
- Allyl 2-methylcrotonate [FL-no: 09.493]
- Allyl cyclohexanepropionate [FL-no: 09.498]
- Pent-2-enyl hexanoate [FL-no: 09.678]
- Allyl phenoxyacetate [FL-no: 09.701]
- Allyl anthranilate [FL-no: 09.719]
- Allyl cinnamate [FL-no: 09.741]
- Allyl phenylacetate [FL-no: 09.790]

- 2-Hexenyl octanoate [FL-no: 09.841]
- Allyl valerate [FL-no: 09.866]
- (E,Z)-2,6-Nonadienyl acetate [FL-no: 09.947]
- (2E)-2-Nonenyl acetate [FL-no: 09.948]
- Allyl 2-furoate [FL-no: 13.004].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the following 74 substances: hex-2-en-1-ol [FL-no: 02.020], nona-2,6-dien-1-ol [FL-no: 02.049], pent-2-en-1-ol [FL-no: 02.050], non-2(trans)-en-1-ol [FL-no: 02.090], non-2(cis)-en-1-ol [FL-no: 02.112], dec-2-en-1-ol [FL-no: 02.137], hex-2(cis)-en-1-ol [FL-no: 02.156], oct-2-en-1-ol [FL-no:02.192], undec-2-en-1-ol [FL-no: 02.210], tr-2, cis-6-nonadien-1-ol [FL-no: 02.231], 2-dodecenal [FL-no: 05.037], nona-2(trans),6(cis)-dienal [FL-no: 05.058], oct-2-enal [FL-no: 05.060], 2-heptenal [FL-no: 05.070], trans-2-nonenal [FL-no: 05.072], hex-2(trans)-enal [FL-no: 05.073], dec-2-enal [FL-no: 05.076], tridec-2-enal [FL-no: 05.078], pent-2-enal [FL-no: 05.102], 2-undecenal [FL-no: 05.109], octa-2(trans),6(trans)-dienal [FL-no: 05.111], 4-methylpent-2-enal [FL-no: 05.114], dodeca-2,6-dienal [FL-no: 05.120], dodec-2(trans)-enal [FL-no: 05.144], hept-2(trans)-enal [FL-no: 05.150], 2-nonenal [FL-no: 05.171], nona-2(trans),6(trans)-dienal [FL-no: 05.172], tetradec-2-enal [FL-no: 05.179], undec-2(trans)-enal [FL-no: 05.184], 2-hexenal [FL-no: 05.189], trans-2-octenal [FL-no: 05.190], trans-2-decenal [FL-no: 05.191], trans-2-tridecenal [FL-no: 05.195], 1,1-diethoxynona-2,6-diene [FL-no: 06.025], 1,1-diethoxyhex-2-ene [FL-no: 06.031], 1,1-dimethoxyhex-2(trans)-ene [FL-no: 06.072], allyl butyrate [FL-no: 09.054], allyl heptanoate [FL-no: 09.097], allyl nonanoate [FL-no: 09.109], allyl octanoate [FL-no: 09.119], allyl undec-10-enoate [FL-no: 09.146], allyl propionate [FL-no: 09.233], allyl hexanoate [FL-no: 09.244], allyl crotonate [FL-no: 09.247], oct-2-enyl acetate [FL-no: 09.276], oct-2(trans)-enyl butyrate [FL-no: 09.277], hept-2-enyl isovalerate [FL-no: 09.303], allyl hexa-2,4-dienoate [FL-no: 09.312], hept-2-enyl acetate [FL-no: 09.385], hex-2(trans)-enyl acetate [FL-no: 09.394], hex-2(trans)-enyl propionate [FL-no: 09.395], hex-2-enyl butyrate [FL-no: 09.396], hex-2-enyl formate [FL-no: 09.397], hex-2-enyl hexanoate [FL-no: 09.398], hex-2-enyl isovalerate [FL-no: 09.399], hex-2-enyl phenylacetate [FL-no: 09.400], allyl 2-ethylbutyrate [FL-no: 09.410], allyl cyclohexanebutyrate [FL-no: 09.411], allyl cyclohexanevalerate [FL-no: 09.469], allyl cyclohexaneacetate [FL-no: 09.482], allyl isovalerate [FL-no: 09.489], allyl cyclohexanehexanoate [FL-no: 09.492], allyl 2-methylcrotonate [FL-no: 09.493], allyl cyclohexanepropionate [FL-no: 09.498], pent-2-enyl hexanoate [FL-no: 09.678], allyl phenoxyacetate [FL-no: 09.701], allyl anthranilate [FL-no: 09.719], allyl cinnamate [FL-no: 09.741], allyl phenylacetate [FL-no: 09.790], 2-hexenyl octanoate [FL-no: 09.841], allyl valerate [FL-no: 09.866], (E,Z)-2,6-nonadienyl acetate [FL-no: 09.947], (2E)-2-nonenyl acetate [FL-no: 09.948] and allyl 2-furoate [FL-no: 13.004] in accordance with Commission Regulation (EC) No 1565/2000.

HISTORY OF FGE.19

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

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

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

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

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

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

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

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

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

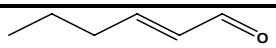
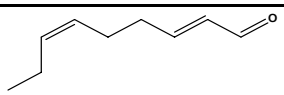
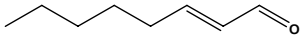
ASSESSMENT

1. History of the Evaluation of the Substances in Subgroup 1.1.1

Subgroup 1.1.1 is one of the FGE.19 subgroups for which the Panel concluded that additional genotoxicity data are needed to perform the safety assessment of the genotoxic potential of the substances (EFSA, 2008a; EFSA CEF Panel, 2011). This conclusion was based on the *in vitro* and *in vivo* genotoxicity data available at that time as well as on the outcome of the (Q)SAR predictions (see Tables 6, 7 and 5, respectively).

Hex-2(trans)-enal [FL-no: 05.073], nona-2(trans),6(cis)-dienal [FL-no: 05.058], oct-2-enal [FL-no: 05.060] and 4,5-epoxydec-2(trans)-enal [FL-no: 16.071] were selected as representative substances to be tested for the subgroup 1.1.1 (EFSA, 2008c). The substance 4,5-epoxydec-2(trans)-enal [FL-no: 16.071] was subsequently considered structurally different from the other substances in subgroup 1.1.1 and was allocated to FGE.226 for evaluation on its own. The representative substances should be tested in accordance with the conditions set out in the "Genotoxicity Test Strategy for Substances belonging to Subgroups of FGE.19" (EFSA, 2008b). The representative substances for subgroup 1.1.1 are shown in Table 1.

Table 1: Representative Substances for Subgroup 1.1.1 of FGE.19 (EFSA, 2008c)

FL-no	EU Register name	Structural formula	Comments
05.073	Hex-2(trans)-enal		Data from literature and new study reports (Beevers, 2013; Bhatia et al., 2010; Dittberner et al., 1995; Dittberner et al., 1997; Durward, 2009; Eder et al., 1992; Griffin and Segall, 1986; Honarvar, 2007a; Kato et al., 1989; Sokolowski, 2007a)
05.058	Nona-2(trans),6(cis)-dienal		Data from literature (Dittberner et al., 1995; Eder et al., 1992)
05.060	Oct-2-enal		Data from literature (Canonero et al., 1990; Eder et al, 1993; Marnett et al.,1985)

In October 2009, the Industry submitted the first dossier in response to the requested data (this dossier was replaced by an updated dossier in April 2010, (EFFA, 2010)).

The Panel considered these new data and its conclusion was given in an EFSA statement published in February 2011 (EFSA CEF Panel, 2011):

"*Supplementary information now provided includes both new data and arguments, which have been discussed by the Panel. Overall, the supplementary information provided by EFFA is not considered sufficient.*

- *Although some of arguments provided by EFFA (e.g. those on metabolism and GSH-depletion and those on the role of DNA damage) are plausible, they are not sufficient to alleviate concerns for the genotoxic and carcinogenic potential of the substances belonging to subgroup 1.1.1.*

- *The data provided are not compliant with the "Genotoxicity Test Strategy for Substances in Subgroups of FGE.19*".

Therefore, the need for additional genotoxicity data has not been alleviated and genotoxicity studies should be carried out for the representative substances of subgroup 1.1.1. In line with the Genotoxicity Test Strategy (EFSA 2008b), the Panel recommended to perform in vivo dietary Comet assays (in drinking water or in feed, not by gavage) for the three linear representatives of subgroup 1.1.1 [FL-no: 05.073, 05.058 and 05.060]. The results may allow to identify whether there is a critical chain length for DNA damage."

FGE	Adopted by EFSA	Link	No. of Substances
Statement on FGE.19 subgroup 1.1.1	21 February 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2086.htm	70
FGE.200	21 May 2014		74

The present Opinion on FGE.200 deals with the additional genotoxicity data submitted by the International Organization of the Flavor Industry (IOFI, 2013) in response to the EFSA statement on the first dossier submitted to EFSA on FGE.200. The data submitted are listed in Table 2.

Furthermore, four additional flavouring substances (*trans*-2,cis-6-nonadien-1-ol [FL-no: 02.231], undec-2(*trans*)-enal [FL-no: 05.184], trans-2-octenal [FL-no: 05.190] and trans-2-tridecenal [FL-no: 05.195]) have been identified which are structurally related to the substances in subgroup 1.1.1 and should be evaluated within this group.

2. Presentation of the Substances Belonging to FGE.200

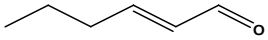
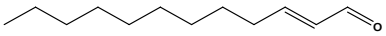
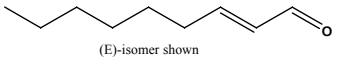
The Flavouring Group Evaluation 200 (FGE.200) concerns 74 straight chain, α,β-unsaturated aldehydes, with or without additional non-conjugated double-bonds, or precursors for such structures. The 74 substances correspond to subgroup 1.1.1 of FGE.19. One former member of subgroup 1.1.1, 4,5-epoxydec-2(trans)-enal [FL-no: 16.071], has been withdrawn from this subgroup and has been evaluated in a new FGE (FGE.226) as the Panel did not consider the substance to be sufficiently structurally related to the other 74 substances in subgroup 1.1.1. The chemical structure of the substances are shown in Table 3 together with their specifications.

3. Additional Data Submitted by Industry for Subgroup 1.1.1

In February 2011, the Panel evaluated the first dossier submitted by the Industry in response to the requested data for representative substances in FGE.200. These data were not considered adequate to alleviate the genotoxicity concern for the substance in subgroup 1.1.1 and concluded: "the Panel recommended to perform *in vivo* dietary Comet assays (in drinking water or in feed, not by gavage) for the three linear representatives of subgroup 1.1.1 [FL-no: 05.073, 05.058 and 05.060]".

In February and June 2013 the Industry (IOFI, 2013) submitted the second dossier which included additional data on one [FL-no: 05.073] of the three representative substances originally selected by the Panel and supporting information to the data already submitted in the first dossier. In Table 2 the newly submitted data are listed.

Table 2: Overview of New Data Submitted for Subgroup 1.1.1 (IOFI, 2013)

Test substance	Test	Test conditions	Reference
Hex-2(trans)-enal [05.073] representative substance (purity: 98.2 %) 	Bacterial reverse mutation assay	*S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 with and without metabolic activation up to 5000 µg/plate.	Sokolowski, 2007a; Bhatia et al.,2010
	In vivo Micronucleus assay	Muta™Mouse blood reticulocytes (days -1, 4 and 31) Treatment by oral gavage at doses of 120, 235 and 350 mg/kg bw/day for 28 days.	Beevers, 2013
	Induction of *lacZ*-mutations in Muta™Mouse	Muta™Mouse treatment by oral gavage at doses of 120, 235 and 350 mg/kg bw/day for 28 days. Mutation frequencies (day 31) determinated in the liver and the duodenum.	Beevers, 2013
	In vivo Micronucleus assay	Treatment by oral route at doses of 250, 500, 1000 mg/kg bw/day. Sampling of bone marrow was done 24 and 48 hours after treatment.	Honarvar, 2007a
	In vivo rat liver unscheduled DNA synthesis (UDS) assay	Treatment by oral route at doses of 200 and 500 mg/kg bw/day. Liver was perfused at 16 and 3 hours after dosing.	Durward, 2009
2-Dodecenal [05.037] not representative (purity: 99.4 %) 	Bacterial reverse mutation assay	*S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 with and without metabolic activation up to 1000 µg/plate.	Sokolowski 2007b; Bhatia et al.,2010
	In vivo Micronucleus assay	Treatment by oral route at doses of 500, 1000 and 2000 mg/kg bw/day. Sampling of bone marrow was done 24 and 48 hours after treatment. 2000 PCEs scored at 24 hours (3 doses) and 48 hours (top dose).	Honarvar, 2007b; Bhatia et al.,2010
2-Nonenal [05.171] not representative (purity: 96.2 %)  (E)-isomer shown	*In vivo* Micronucleus assay	Treatment by oral route at doses of 500, 1000 and 2000 mg/kg bw/day. Sampling of bone marrow was done 24 and 48 hours after treatment. 2000 PCEs scored at 24 hours (3 doses) and 48 hours (top dose).	Honarvar, 2008; Bhatia et al.,2010

3.1. *In vitro* Genotoxicity Tests

Bacterial reverse mutation assays

Hex-2(trans)-enal [FL-no: 05.073]

Hex-2(*trans*)-enal (purity: 98.2 %) was tested at concentrations up to 5000 µg/plate (but concentrations higher than 200 µg/plate were bacteriostatic) in the *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, in a GLP study performed according to OECD Guideline 471 (OECD, 1997a), with or without metabolic activation (Sokolowski, 2007a; Bhatia et al., 2010). A small but concentration-dependent increase in revertant colony numbers was observed using the pre-incubation method in strain TA100 without metabolic activation (concentrations tested 1 - 2500 µg/plate). Toxic effects at higher concentrations reduced the number of revertants. Smaller increases (< 2-fold) were also seen in the presence of S9-mix. Therefore, a follow-up experiment, again using the pre-incubation method, was performed in strain TA100 over a narrow range of concentrations up to 200 µg/plate. In this follow-up experiment, a moderate concentration-dependent increase in revertant colony numbers was again observed without metabolic activation at 50 and 100 µg/plate. Based on the reproducibility of this effect, the author concluded a positive mutagenic outcome for this test. While the magnitude of the increase in revertant colony numbers is not substantial, these results do not exclude possible mutagenic potential in strain TA100 (Sokolowski, 2007a).

Kato et al. (1989) tested hex-2(*trans*)-enal (unknown purity) in the *S. typhimurium* strains TA98, TA100, and TA104 and in *Escherichia coli* strain WP2uvrA/pKM101 with and without metabolic activation using the pre-incubation method (20 min at 37 °C). According to the authors, hex-2(*trans*)-enal was 'suspected to be positive'; however, no further details were provided and the validity of this study is limited.

2-Dodecenal [FL-no: 05.037]

At concentrations up to 1000 µg/plate with and without metabolic activation (but concentrations ≥ 100 µg/plate were bacteriostatic) 2-dodecenal (purity: 99.4 %) was not mutagenic in the *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, in a GLP study performed according to OECD Guideline 471; the limiting factor was the bacteriostatic activity (Sokolowski, 2007b). Toxic effects (reduction in revertant numbers) were seen at the higher concentrations in all parts of the study. No genotoxic effect was noted with and without metabolic activation in the five strains.

The same data for the bacterial reverse mutation assay reported by Sokolowski (2007a, b) for hex-2(trans)-enal [FL-no: 05.073] and 2-dodecenal [FL-no: 05.037] were presented in a poster abstract (Bhatia et al., 2010).

Summary of the bacterial reverse mutation assays for both hex-2(*trans*)-enal [FL-no: 05.073] and 2-dodecenal [FL-no: 05.037] are reported in Table 8.

3.2. *In vivo* Genotoxicity Tests

Hex-2(trans)-enal [FL-no: 05.073]

On the basis of the *in vitro* bacterial reverse mutation assay results reported above for hex-2(*trans*)-enal, it was considered most appropriate to probe its genotoxic potential using a Muta™Mouse (lacZ/GalE) assay with an *in vivo* micronucleus component included (Beevers, 2013). The assay was carried out in transgenic mice. This combined approach minimises the number of animals used in the experiments. Micronuclei were measured in peripheral blood, and in the mutation arm of the experiment, the liver and the duodenum were chosen as the most appropriate tissues, in order to

address the potential for mutation at the site of most significant metabolism and at the site of first contact, respectively. Therefore, groups of Muta™Mouse CD2-lacZ80/HazfBR mice were administered hex-2(*trans*)-enal via gavage and the liver, duodenum and peripheral blood were analysed for the potential induction of DNA damage in a GLP study performed according to OECD Guidelines 474 (OECD, 1997b) and 488 (OECD, 2011). However, the Panel noted that there were some deviations from OECD guideline 474 (see below and Table 9).

An initial Range-Finder study was conducted to estimate the maximum tolerated dose (MTD) of hex-2(*trans*)-enal (purity 99.5 %) after administration by oral gavage to groups of three male and three female Muta™Mouse mice. Doses of 500 mg/kg body weight (bw)/day were clearly toxic to mice, with 1 animal being killed in extremis on day 4 and the rest of the animals exhibiting signs of toxicity (piloerection, hunched posture) but surviving to day 7. Further groups of animals were also dosed at 250 and 350 mg/kg bw/day. No clinical signs of toxicity were observed at 250 mg/kg bw/day, but at 350 mg/kg bw/day 1 animal showed signs of clinical toxicity (hunched posture, decreased activity and dyspnoea). As a result, 350 mg/kg bw/day was identified as the MTD. As no significant gender differences in clinical signs of toxicity were observed, it was concluded that male mice alone could be used in the main experiment. Two lower doses of 120 and 235 mg/kg bw/day were also selected for testing.

Groups of six male Muta™Mouse mice were treated daily by oral gavage with hex-2(*trans*)-enal at doses of 120, 235 and 350 mg/kg bw/day, including a vehicle control (corn oil) for 28 days with a 3-day recovery period prior to sacrifice. Concurrent positive control animals were not included in this study. Tissue matched positive control DNA was included in all packaging reactions in order to confirm correct assay functioning. The positive control DNA originated from animals dosed with ethylnitrosurea. All individual packaging reaction resulted in at least 30 000 plaque-forming unit (PFU) and at least 1 mutant plaque. For all animals data were generated for at least 200 000 PFU per tissue, from at least three independent packaging reactions. At least 1 million PFU were obtained per group, per tissue from a minimum of five animals. No significant increases in mutation frequency (MF) or significant dose-related trends were observed in the liver or the duodenum. Some of the hex-2(*trans*)-enal treatment groups showed duodenum MF that exceeded laboratory historical controls but were comparable to concurrent vehicle control values. The testing laboratory had a limited number of datasets that comprise the historical control data for the duodenum in this assay and considered its historical control for the duodenum in the Muta^{TM}Mouse assay to be narrow at the time of drafting this report.

Hex-2(*trans*)-enal was evaluated in a micronucleus assay in peripheral normochromatic erythrocytes and reticulocytes for its ability to induce chromosomal damage (micronuclei, MN) in mice on days 4 and 31 after 28 days of dosing, using a flow cytometry method. Where possible, 20 000 reticulocytes were analysed from each blood sample. No significant differences were observed in the frequency of peripheral blood reticulocytes (% RET) in all treatment groups on day 4 or 31 after 28 days of dosing. There were no significant increases in the frequency of micronuclei compared to concurrent controls on day 4 or 31 after 28 days of dosing. On day 31, it was noted that there was a significant linear trend in micronucleated reticulocyte (% MN-RET) frequency ($P \leq 0.05$); however, as the MN-RET frequencies for all treated animals (0.37 ± 0.04, 0.39 ± 0.05, 0.39 ± 0.06, 0.46 ± 0.09 at doses of 0, 120, 235, 350 mg/kg bw/day respectively) were highly consistent with the Day -1 background levels of MN-RET (0.38 ± 0.04, 0.39 ± 0.05, 0.41 ± 0.05, 0.42 ± 0.05, at doses of 0, 120, 235, 350 mg/kg bw/day respectively), the significant linear response was considered to be an artefact and was not indicative of any accumulation of micronuclei over time (Beevers, 2013).

The Panel noted that in the micronucleus arm of the study, the peripheral blood was sampled 72 hours after the treatment while the OECD Guideline 474 recommends: ”once between 36 and 48 hours following the final treatment for the peripheral blood”. This point limited the reliability of the results obtained in the micronucleus part of the assay.

Hex-2(*trans*)-enal (purity: 98.2 %) was evaluated in a micronucleus assay in bone marrow polychromatic erythrocytes (PCEs) for its ability to induce chromosomal damage (micronuclei, MN) in mice in a GLP study performed according to OECD Guideline 474 (OECD, 1997b). Hex-2(*trans*)-enal dissolved in corn oil as a carrier was given orally to animals (5 males and 5 females) at doses of 250, 500, and 1000 mg/kg bw. The high dose was determined in a preliminary toxicity study. Mice from all dose groups were sampled 24 hours after dosing, and mice from top dose and control groups were also sampled 48 hours after dosing (Honarvar, 2007a).

Cyclophosphamide (40 mg/kg bw) was given as the positive control and mice were sampled at 24 hours. At least 2000 PCEs were scored for each animal for MN. At the highest dose given, 2 males and 2 females died, which indicates that higher doses could not have been used. Also in the highest dose group the numbers of PCEs were clearly decreased (-35 % at 24 hours) as compared to the mean value of PCEs of the vehicle control. This indicates that hex-2(*trans*)-enal exerts cytotoxic effects in the bone marrow at this dose level and demonstrates, in the absence of toxicokinetic measures, that the target tissue was exposed. In comparison to the corresponding vehicle controls there was no statistically significant increase in the frequency of the detected micronuclei at any preparation interval after administration of the test item with any dose level used (Honarvar, 2007a).

2-Hexenal (unspecified isomer and purity) was evaluated in an *in vivo* unscheduled DNA synthesis assay using oral administration in a GLP study performed according to OECD Guideline 486 (OECD, 1997c) (Durward, 2009). Male rats were given 200 or 500 mg/kg bw 2-hexenal. The top dose was proposed by the sponsor, and a preliminary test by the testing facility demonstrated no deaths at this dose. As no other dose levels were used, it is not clear that this was the maximum tolerated dose, and perhaps a higher dose could have been used. In one experiment, livers were perfused approximately 16 hours after dosing and in a second experiment 3 hours after dosing. Following perfusion, hepatocytes were processed and areas of nucleus and cytoplasm scored for autoradiographic grains in 150 cells/animal at each sampling time using automated image analysis. A control group was given only corn oil, and positive control groups were administered 2-acetylaminofluorene (16 hours) or N,N'-dimethylhydrazine (3 hours). Net nuclear grain counts were < 0 at the two harvest times and the percentage of cells in repair was low in all animals dosed with 2-hexenal at the 3-hour harvest time. The percentage of cells in repair at the 16-hour harvest time was weakly increased with 1.8 ± 1.7 % and 2.2 ± 0.6 % cells in repair at 200 and 500 mg/kg respectively vs. 0.4 ± 0.6 % in the concurrent control, however, these values are low and within those generally observed. In the absence of an increase in the number of net grain per cell, these variations have no meaning in term of genotoxic effect. There was therefore no evidence of induction of unscheduled DNA synthesis in animals dosed with the test material at either time point.

2-Dodecenal [FL-no: 05.037]

2-Dodecenal (purity: 99.4 %) was evaluated in a micronucleus assay in bone marrow PCEs for its ability to induce chromosomal damage in mice in a GLP study performed according to OECD Guideline 474 (OECD, 1997b). 2-Dodecenal, dissolved in corn oil as a carrier, was given orally to animals (5 males and 5 females) at doses of 500, 1000 and 2000 mg/kg bw. The top dose of 2000 mg/kg bw is a limit dose for non-toxic substances. Mice from all dose groups were sampled 24 hours after dosing, and mice from top dose and control groups were sampled also at 48 hours after dosing. Cyclophosphamide (40 mg/kg bw) was given as the positive control and mice were sampled at 24 hours. At least 2000 PCEs were scored for each animal for MN. No cytotoxic effects were observed at any dose, based on the ratio between PCEs and NCEs in each treated sample versus vehicle controls.

In comparison to the corresponding vehicle controls there was no statistically significant increase in the frequency of the detected micronuclei at any preparation interval after administration of the test item with any dose level used (Honarvar, 2007b).

2-Nonenal [FL-no: 05.171]

2-Nonenal (purity: 96.2 %) was evaluated in a micronucleus assay in bone marrow PCEs for its ability to induce chromosomal damage in mice in a GLP study performed according to OECD Guideline 474 (OECD, 1997b). 2-Nonenal, dissolved in corn oil as a carrier, was given orally to animals (5 males and 5 females) at doses of 500, 1000, and 2000 mg/kg bw. The top dose of 2000 mg/kg was estimated as suitable by a preliminary study on acute toxicity. Mice from all dose groups were sampled 24 hours after dosing, and mice from top dose and control groups were sampled also at 48 hours after dosing. Cyclophosphamide (40 mg/kg bw) was given as the positive control and mice were sampled at 24 hours. At least 2000 PCEs were scored for each animal for MN. The numbers of PCEs were slightly decreased, mainly in the top dose group at both sampling times, as compared to the mean value of PCEs of the vehicle control (-13 % at 24 and 48 hours sampling times). However, the decrease in % PCE was small. In comparison to the corresponding vehicle controls there was no statistically significant increase in the frequency of the detected micronuclei at any preparation interval after administration of the test item with any dose level used (Honarvar, 2008).

For both 2-dodecenal and 2-nonenal tested through micronucleus assays in mouse bone marrow PCE (Honarvar, 2007b; Honarvar, 2008) there was no direct confirmation that the bone marrow was exposed, as no toxicokinetic measures of the test substance in plasma were made.

Micronucleus data for hex-2(*trans*)-enal (Hornavar 2007a), 2-nonenal (Honarvar, 2008) and 2-dodecenal (Honarvar, 2007b) were reported also in a poster abstract (Bhatia et al., 2010).

The results of *in vivo* studies are summarised in Table 9.

3.3. DNA Adduct and Related Studies

DNA adduct studies in vitro

The ability of the α,β-unsaturated aldehydes to bind to isolated nucleosides and nucleotides *in vitro* has been reported (Stout et al., 2008; Eisenbrand et al., 1995; Golzer et al., 1996; Eder et al., 1993). 2-Hexenal and related α,β-unsaturated aldehydes are capable of forming 1,N^2-cyclic deoxyguanosine and 7,8-cyclic guanosine adducts.

DNA adduct studies in vivo on hex-2(trans)-enal [FL-no: 05.073]

Using a ^{32}P-post-labelling method based on nuclease P1 enrichment and TLC separation of the labelled adducts[7], *in vivo* studies on hex-2(*trans*)-enal report adducts formation. In a first study (Schuler et al., 1999) administered hex-2(*trans*)-enal at a single dose of 500 mg/kg bw by oral route to F344 male rats. No adducts were found in the control rats. In treated rats, an adduct (1,N^2-propanodeoxyguanosine (Hex-PdG)) was detected in the liver. Highest Hex-PdG adduct levels were found 2 days after gavage. Four days after gavage, the Hex-PdG adducts level was one third of the maximum level but it was even higher than Hex-PdG adducts found after 1 day. No adducts were detected 8 hours after gavage. This study demonstrates that after one single high dose of hex-2(*trans*)-enal, formation of DNA adducts were induced, that there was a delay before apparition of adducts in the liver and that these adducts were repaired only slowly.

Schuler and Eder (1999) detected Hex-PdG adducts in the forestomach, liver, esophagus and kidneys of F344 rats at relatively high single doses, i.e., 200 and 500 mg/kg bw of hex-2(*trans*)-enal by gavage. At 50 mg/kg bw Hex-PdG adducts were quantified only in the esophagus. The covalent binding index was 0.06, 0.22 and 0.62 at 50, 200 and 500 mg/kg bw respectively (Schuler and Eder, 1999).

[7] detection limit 0.03 adducts per 10^6 nucleotides

In the study performed by Stout and colleagues (Stout et al., 2008), using a LC/MS/MS[8] method, no adduct formation was reported at 50 mg/kg bw of hex-2(*trans*)-enal except in forestomach DNA of one rat exposed to a single dose and sacrificed 2 days after (Stout et al., 2008). Quantifiable levels of Hex-PdG adducts were reported in the forestomach of animals exposed to 100 mg/kg bw/day of hex-2(*trans*)-enal for 1 or 4 weeks (once daily for 5 days per week) and at 200 mg/kg bw of hex-2(*trans*)-enal in single doses. However, Hex-PdG was not quantifiable in forestomach DNA of rats after exposure to 0, 10 or 30 mg/kg for 1 or 4 weeks (Stout et al., 2008). These data are indicative of a dose- and time-dependence on DNA adducts formation with hex-2(*trans*)-enal. Hex-PdG was not quantifiable in liver DNA after exposure to 100 mg/kg for 1 or 4 weeks. These findings suggest that the genotoxicity of hex-2(*trans*)-enal was limited to the site of contact (forestomach) and DNA adduct formation occurred in the setting of severe tissue damage as demonstrated by histopathological observations. At these cytotoxic doses, cell proliferation was noted. The Panel noted that no DNA adducts were observed at 30 mg/kg/day and below.

3.4. Data on toxicokinetic

Analogous to other α,β-unsaturated aldehydes, *trans*-2-hexenal is readily oxidized *in vitro* to trans-2-hexenoic acid in the cytosolic fraction of mouse liver cells (Lame and Segall, 1986) and by isoenzymes of rat aldehyde dehydrogenase (ALDH) present in mitochondrial, cytosolic, and microsomal fractions (Mitchell and Petersen, 1987). In general, the members of the ALDH superfamily demonstrate higher catalytic activity *in vitro* for higher molecular weight and more lipophilic aldehydes (Nakayasu et al., 1978).
Prior to absorption, 15% of a 100 mg/kg bw dose of trans-2-nonenal given to rats was oxidized to trans-2-nonenoic acid (Grootveld et al., 1998).
Linear α,β-unsaturated aldehydes are rapidly absorbed, distributed, metabolized and excreted in the urine and, to a lesser extent, in the faeces. In *in vivo* experiments with trans-2-nonenal and trans-2-pentenal, male Wistar albino rats were administered a bolus dose of 100 mg/kg bw of one of the aldehydes by gavage in unheated olive oil. A control group of rats received only the unheated olive oil. Urine samples were collected prior to and after administration. ^{1}H-NMR analysis indicated that both trans-2-nonenal and trans-2-pentenal entered systemic circulation from the gastrointestinal tract and were metabolized in the fatty acid pathway or were conjugated with glutathione to yield the C-3 mercapturate conjugate that is excreted mainly in the urine within 24 hours. Trace amounts of trans-2-nonenal and trans-2-pentenal were detected in the faeces (Grootveld et al., 1998).

PBK/D model

A recent Physiologically-Based Kinetic/Dynamic (PBK/D) study supports a dose-dependent effect on hex-2(*trans*)-enal detoxification and development of DNA adducts (Kiwamoto et al., 2012, 2013). The detoxification of trans-2-hexenal proceeds via three pathways: oxidation to 2-hexenoic acid by aldehyde dehydrogenase (ALDH), reduction to 2-hexen-1-ol by aldose reductase (AR), conjugation with reduced glutathione (GSH) either chemically or catalysed by glutathione S-transferase (GST) (Eisenbrand et al., 1995). Kiwamoto et al. (2012) developed a PBK/D model in rats determining *in vitro* kinetic parameters (e.g. Km, Vmax and catalytic efficiency) for each detoxification pathway. Performance of the model was evaluated against available *in vivo* data from literature on rats exposed to high doses of trans-2-hexenal (Shuler and Eder, 1999; Stout et al., 2008). In this study, it was shown that when hex-2(*trans*)-enal is incubated with S9-mix fractions of rat liver and rat small intestine, in the presence of NAD^{+}, both fractions predominantly convert the substrate to 2-hexenoic acid which does not readily form DNA conjugates and is efficiently eliminated from the urine in the form of glucuronic acid conjugates. This model predicts that the conversion of trans-2-hexenal at doses of 0.04 mg/kg bw (predicted human dietary exposure) and 200 mg/kg bw (dose at which DNA adduct formation in the liver was reported in rats, by Shuler and Eder, 1999) is complete within 3 hours. At 0.04 mg/kg bw GSH concentration is not affected both in liver and small intestine. At 200 mg/kg bw

[8] The limit of quantitation was 0.015 fmol Hex-PdG/µg DNA (200 µg DNA) or 0.006 fmol Hex-PdG/µg DNA (500 µg DNA).

GSH concentration in the small intestine (predicted as the most important detoxification pathway in this tissue) dropped rapidly and amounted to only 65% of the initial level after 24 hours; also in the liver GSH concentration is depleted, but restored within 24 hours. The model suggests that at low doses of trans-2-hexenal, protective levels of GSH are unaffected, while at high doses significant GSH depletion occurs. The model predicts that at doses below 80 mg/kg bw all the three pathways contribute to trans-2-hexenal detoxification in the liver. The PBK/D model predicts that hex-2(*trans*)-enal is readily detoxified through glutathione conjugation at 30 mg/kg bw and below. The same model was further developed to examine dose-dependent detoxification and DNA adducts formation in humans upon dietary exposure (Kiwamoto et al., 2013). In this study the kinetic parameters were derived from literature or calculated through *in vitro* reactions using human tissue fractions, taking into account interindividual differences. The model reveals that rapid *in vivo* detoxification of hex-2(*trans*)-enal at levels of average dietary exposure (0.04 mg/kg bw) makes DNA adduct formation negligible (Kiwamoto et al., 2013). Additionally, EFFA estimated a daily exposure of 0.01 mg/kg bw/day for hex-2(*trans*)-enal (EFFA, 2010) which is below the concentrations predicted to induce DNA adduct formation.

The Panel noted that all the metabolic parameters were obtained from *in vitro* studies using rat (Kiwamoto et al., 2012) or human (Kiwamoto et al., 2013) liver S9-mix or small intestine S9-mix and cofactors or liver mitochondrial fraction to determine the kinetic constants for ALDH-mediated oxidation, AR-mediated reduction and GST-catalysed conjugation of GSH with *trans*-2-hexenal in these different tissue fractions. Due to the fact that data were obtained only *in vitro*, such a model is limited. The Panel noted that for these reasons, this model should be considered with cautions.

3.5. Discussion of Mutagenicity/Genotoxicity and Related Relevant Data

In Ames assays, positive results in TA100 and TA104, were reported for several of the substances in subgroup 1.1.1, particularly when pre-incubation conditions were used. Slight concentration-dependent increase in revertant colony numbers was observed with hex-2(*trans*)-enal [FL-no: 05.073] and pent-2-enal [FL-no: 05.102] but not with nona-2(*trans*),6(*cis*)-dienal [FL-no: 05.058] and 2-octenal [FL-no: 05.060]. When using a three-fold bacterial cell density, pent-2-enal and hex-2(*trans*)-enal were clearly mutagenic with and without metabolic activation; hept-2(*trans*)-enal induced a weak and concentration-dependent mutagenic effect with metabolic activation and it was clearly mutagenic without S9-mix. In this assay it was demonstrated that mutagenicity decreased and toxicity increased with increasing length of the alkyl chain in β-position (Eder et al., 1992). The authors suggested that the dependence of cell toxicity on the increasing β-chain length could be related to the increasing lipophilicity. A double bound in the β-alkyl chain conjugated with that of the acrolein moiety exerted a special effect: it increases the mutagenicity significantly (Eder et al., 1992). This has been confirmed in recent GLP studies (Sokolowski, 2007a; Sokolowski, 2007b).

Five alk-2-enals: penta-2-enal, hex-2-enal, hept-2-enals, oct-2-enal and non-2-enal (isomers not specified) were tested for mutagenic activity in V79 Chinese hamster cells. All five alk-2-enals induced a concentration-dependent increase of 6-thioguanine (TG) resistant mutants with a statistically significant increase at 0.3 mM for penta-2-enal and hex-2-enal, at 0.1 mM for hept-2-enal and oct-2-enal and at 0.01 mM for non-2-enal. The authors reported that a significant increase in mutation frequency is caused by alkyl-2-enal concentrations that caused cytotoxicity. Both mutagenicity and cytotoxicity seems directly related to the chain length of the compound. Only hept-2-enal induced a statistically significant increase in the frequency of mutations to ouabaine resistance in the same cell line (Canonero et al., 1990). This study was considered of limited validity because there is no information about the cytotoxicity levels at each concentration tested, the number of tested concentrations is limited (2 or 3) and the criteria for their choices not clearly presented.

Hex-2(*trans*)-enal [FL-no: 05.073] and *trans*-2-nonenal [FL-no: 05.072] were positive in an *in vitro* UDS assay performed in primary cultures of rat hepatocytes. Concentrations of both compounds from 60 to 600 nmol/10^6 cells (equal to 70 nmol/ml to 700 nmol/ml) showed a concentration-dependent increase of cells positive for UDS (Griffin and Segall, 1986).

In the study by Eder et al. (1992), it is shown that in the presence of S9-mix there is a shift in toxicity toward higher chemical concentrations, suggesting that S9-mix could lead to partial detoxification. Also Marnett et al. (1985) reported that toxicity is an important factor in the detection of enals as mutagens. The authors observed positive results only in the presence of glutathione and attributed this effect to a partial detoxification that allows survival of bacteria and the growth of revertant colonies.

In the TA104 strain (which carry one non-sense mutation TAA in the main DNA and not on a plasmid like TA102 strain), 2-hexenal [FL-no: 05.189] was mutagenic, but 2-heptenal [FL-no: 05.070], 2-octenal [FL-no: 05.060] and 2-nonenal [FL-no: 05.171] were not mutagenic. No mutagenic activity was observed in the TA102 strain (Marnett et al., 1985).

Positive evidence of genotoxicity was also reported in other assays (sister chromatid exchange (SCE), chromosomal aberrations (ABS), micronuclei (MN), hypoxanthine guanine ribosyl transferase (HPRT) mutations, and unscheduled DNA synthesis (UDS)) in mammalian cells, but more particularly in cell lines that have low detoxification capacity e.g., Namalva cells and V79 cells (Esterbauer et al., 1990; Eckl et al., 1993; Canonero et al., 1990; Griffin and Segall, 1986).

Hex-2(*trans*)-enal [FL-no: 05.073] (concentrations tested from 5 to 250 μM) and nona-2(*trans*),6(*cis*)-dienal [FL-no: 05.058] (concentrations tested from 5 to 40 or 50 μM) were tested in a human lymphoblastoid Namalva cell line and in human lymphocytes for SCE, ABS and MN induction without metabolic activation. Both aldehydes increased the frequency of SCE in the two cell types. The treatment with hex-2(*trans*)-enal induced a statistically significant increase in SCE from 40 μM on lymphocytes and 20 μM for Namalva cells. Nona-2(*trans*),6(*cis*)-dienal induced a statistically significant increase in SCE from 20 μM on lymphocytes and 10 μM for Namalva cells. Nona-2(*trans*),6(*cis*)-dienal was more cytotoxic than hex-2(*trans*)-enal. In human lymphocytes, neither hex-2(*trans*)-enal nor nona-2(*trans*),6(*cis*)-dienal induced statistically significant increase of structural chromosome aberrations. On the contrary, in Namalva cells, both hex-2(*trans*)-enal and nona-2(*trans*),6(*cis*)-dienal induced structural chromosome aberrations from 100 μM and 5 μM respectively. Hex-2(*trans*)-enal and nona-2(*trans*),6(*cis*)-dienal induced aneuploidies in human lymphocytes from 40μM. Hex-2(*trans*)-enal increased the frequencies of micronuclei both in lymphocytes and in Namalva cells in a concentration-dependent manner. The increase of MN frequency, induced by hex-2(*trans*)-enal, was statistically significant in lymphocytes from 50 μM and in Namalva cells from 150 μM, while for nona-2(*trans*),6(*cis*)-dienal a statistically significant increase of MN frequency was observed from 20 μM in lymphocytes and from 40 μM in Namalva cells. Using fluorescent *in situ* hybridisation, both lymphocytes and Namalva cells showed significantly enhanced frequencies of centromere positive micronuclei for both hex-2(*trans*)-enal and nona-2(*trans*),6(*cis*)-dienal, which is coherent with the observation of aneuploidy inductions in the cytogenetic assay. This study shows that both hex-2(*trans)*-enal and nona-2(*trans*),6(*cis*)-dienal gave equivocal results in lymphocytes and positive results in Namalva cells, for structural aberrations. While for aneugenicity hex-2(*trans)*-enal and nona-2(*trans*),6(*cis*)-dienal were positive in both cell types. The Namalva cells were generally more sensitive than lymphocytes. These cells have been found poor or even totally deficient in many detoxifying enzymes and they also contain only rather low concentrations of glutathione and of glutathione-related enzymes (Dittberner et al., 1995).

Using an alkaline elution method, Eisenbrand et al., (1995) demonstrated that Namalva cells were significantly more sensitive than primary rat hepatocytes to the induction of DNA strand breaks by hexenal. In hepatocytes about 3-5 times higher concentrations of aldehydes were necessary to induce significant effects compared to Namalva cells. The authors explained this difference by the better enzymatic activity (GSH transferase, aldehyde dehydrogenase) in primary rat hepatocytes compared to Namalva cells. In this study, the authors demonstrated that hexenal induced DNA binding in a range of doses from 1 to 5 mM (Eisenbrand et al., 1995).

Dittberner et al. (1997) performed studies on exfoliated cells of human oral mucosa. Seven healthy non-smoking volunteers rinsed their mouth 4 times per day for 3 days with 100 ml of hex-2(*trans*)-enal [FL-no: 05.073] solution at the concentration of 10 ppm, which represents a possible

concentration in food. Results showed at least a doubling of micronuclei frequency in exfoliated cells of human oral mucosa during one of the next four days, then the MN number dropped down to nearly the control level. In a second study, seven other volunteers were observed before and after eating 3 - 6 bananas that contained 35 ppm hex-2(*trans*)-enal. Six of the seven volunteers showed at least a doubling of the MN frequency during one of the next six days (Dittberner et al., 1997). The Panel noted that the results were statistically significant and that the protocol was consistent with standard protocols recently developed for biomonitoring studies. Therefore, the results are considered reliable. However, the Panel also noted that this kind of studies is not validated for regulatory purposes.

Primary rat hepatocytes were treated for 3 hours with 0, 0.1, 1.0, 10 and 100 µM of *trans*-2-nonenal [FL-no: 05.072] followed by a 48 hours recovery period. *trans*-2-Nonenal induced an increase ($p < 0.01$) in micronuclei at 10 and 100 µM. At a concentration of 100 µM the mean value of chromosomal aberrations was 2.7-fold higher than in the controls, but due to the high standard deviations, these increases were not statistically significant (Esterbauer et al., 1990).

Primary rat hepatocytes were seeded and after 20 hours treated with *trans*-2-nonenal [FL-no: 05.072] at 0, 0.1, 1, 10 or 100 µM for 3 hours. Then the culture medium was replaced by fresh medium added with EGF and BrdU (Eckl et al., 1993). 48 hours after the end of the treatment, cells were treated with colcemid, and sampled 3 hours later. Slides treated with Hoechst 33258 were used for determination of SCE and the other for chromosomal aberration. *trans*-2-Nonenal induced no significant toxicity at the highest concentration tested. *trans*-2-Nonenal increased neither chromosomal aberrations nor the frequency of micronuclei. The Panel noted that in this study, EGF was added to induce cell division, but cells were not in division during the period of treatment, this deviation could result in a bias compared to recommended protocols. The Panel noted that cells used to determine the induction of chromosomal aberrations and micronuclei were pre-treated with BrdU which weakens the chromosomes. The testing for chromosomal aberrations and micronuclei was done after a short treatment, followed by a long recovery time which does not appear to be an optimum protocol and is a deviation from the OECD Guidelines. Moreover, hepatocytes do not divide all since the mitotic index in control cultures ranged from 0.41 to 1.94 %, and no method (such as the addition of cytochalasin B) was used to determine the frequency of micronuclei only in cells that divided which reduces the sensitivity of this test (Eckl et al., 1993).

Chung et al. (1999) reported that formation of cyclic propano adducts are common products from reactions of enals with DNA bases. Enals derive from lipid peroxidation of cell membrane, but the contribution from environmental sources, cannot be excluded. The mutagenicity of enals and the mutations observed in site-specific mutagenesis studies, using a model for $1,N^2$-propanodeoxyguanosine adducts, suggest that these adducts are potential promutagenic lesions. The authors showed that tissue GSH plays an important role in protecting DNA from cyclic adduction by enals.

Coles and Ketterer (1990) reported that 4-hydroxynon-2-enal is a substrate of different classes of rat glutathione transferases that detoxified this compound. But the authors concluded that these enzymes do not provide a perfect protection and cytotoxic or genotoxic damage cannot always be avoided.

Kelson et al. (1997) isolated and characterized a human microsomal fatty aldehyde dehydrogenase, which is a distinct human aldehyde dehydrogenase isozyme that acts on a variety of medium- and long-chain aliphatic substrates with a high activity towards saturated and unsaturated aliphatic aldehydes ranging from 6 to 24 carbons in length.

In cell lines poor in detoxification capacity, there is an opportunity for high concentrations (20 to 40 µM) of α,β-unsaturated aldehydes to either interact directly with DNA or indirectly forming DNA adducts due to oxidative stress, leading to single DNA strand breaks but no cross-linking of DNA. The depletion of GSH by high concentrations of α,β-unsaturated aldehydes is known to lead to oxidative stress and to the release of nucleocytolytic enzymes, causing DNA fragmentation, cellular damage and apoptosis (see sections 3.3 and 3.4). Hex-2(*trans*)-enal, 2-nonenal and 2-dodecenal did not induce MN

in mice in robust GLP studies (Honarvar, 2007a, 2007b, 2008). However, only for hex-2(*trans*)-enal exposure of the target tissue was demonstrated. In addition, hex-2(*trans*)-enal did not induce unscheduled DNA synthesis in rat hepatocytes at doses up to 500 mg/kg bw in a GLP study (Durward, 2009).

However, this type of assays does not address the potential clastogenicity in the gastrointestinal tract. While such studies have not been conducted, due to structure similarity it seems to be possible that lifetime gavage administration of high concentrations of hex-2(*trans*)-enal [FL-no: 05.073] to rats might result in carcinogenicity in the forestomach or esophagus, similar to that observed for 2,4-hexadienal (subgroup 1.1.4 of FGE.19, EFSA CEF Panel, 2014). It is also likely that the ulcerative and necrotising lesions and consequent regenerative cell proliferation in the forestomach produced under these unique conditions would be associated with increased DNA adducts, as was observed in the hexenal DNA adduct study (Stout et al., 2008). However, production of exocyclic guanine adducts following glutathione depletion may be involved, but the evidence from the 2-hexenal and 2,4-hexadienal studies suggests that these events are associated with significant tissue damage (ulceration, inflammation and hyperplasia) related to high bolus dosing by gavage. The inflammation and tissue damage could affect the normal biochemical processes involved in the metabolism of α,β-unsaturated aldehydes and their detoxification. The reduced metabolic activity could increase the probability of a direct reaction between aldehydes and DNA nucleotides.

A recent PBK/D model shows that 2-hexenal is rapidly detoxified predominantly by conjugation with glutathione (GSH) by glutathione S-transferases, and that the rapid detoxification of 2-hexenal reduces the risk arising from 2-hexenal exposure through the diet. Thus, dietary exposure to doses that do not deplete glutathione, and therefore do not lead either to tissue damage or DNA adducts would not be expected to pose a mutagenic or carcinogenic hazard (Kiwamoto et al., 2012, 2013). However, the Panel considered that PBK/D studies are not sufficient due to the lack of validation.

Hex-2(*trans*)-enal induced weak gene mutations in bacteria. When tested in the Muta™Mouse assay up to the maximum tolerated dose of 350 mg/kg bw/day, hex-2(*trans*)-enal was not mutagenic in the tissues of the duodenum, presumably the first point of contact for the test material upon transit from the glandular stomach. These results are also supported by no indication of mutagenic activity in the liver, primary point of metabolism.

Hex-2(*trans*)-enal tested for chromosomal aberrations in mammalian cell lines showed positive results, but resulted negative in the *in vivo* micronucleus test performed in peripheral blood reticulocytes (Beevers, 2013) and in bone marrow (Honavar, 2007a).

In summary, the available data indicate that at high concentrations of hex-2(*trans*)-enal no gene mutations were induced in the liver and duodenum of transgenic mice after a daily treatment for 28 days up to 350 mg/kg bw/day (Beevers, 2013). DNA adducts were detected in the forestomach, liver, esophagus and kidneys of rats treated with hex-2(*trans*)-enal by gavage at relatively high single doses, i.e., 200 and 500 mg/kg bw and at 50 mg/kg in the esophagus. DNA adducts were not quantifiable in forestomach DNA of rats after exposure to 10 or 30 mg/kg bw for 1 or 4 weeks (Schuler and Eder, 1999). However, in the same experimental condition, DNA adducts were detected locally (duodenum and esophagus) and systemically (kidney and liver) at doses lower than the dose that proved no induction of gene mutation. The Panel noted that overall the available experimental data from animals and humans, while not showing an induction of gene mutations, do not allow to assess the potential clastogenic activity of hex-2(*trans*)-enal at the first site of contact and in the liver, where high levels of DNA adducts were observed.

CONCLUSION

The new data submitted are related only to one representative substance of subgroup 1.1.1, hex-2(*trans*)-enal [FL-no: 05.073].

For hex-2(*trans*)-enal [FL-no: 05.073] gene mutations were observed *in vitro* in TA100, and chromosomal aberrations were observed *in vitro* likewise. In addition, a biomonitoring study in human buccal cells showed a statistically significant increase in the frequency of micronuclei at concentrations that might be relevant for the use of hex-2(*trans*)-enal as flavouring substances. The new submitted study performed on a Muta^{TM}Mouse model does not cover these endpoints adequately. The Panel noted that overall the available experimental data from animals and humans, while not showing an induction of gene mutations, do not allow to assess the potential clastogenic activity of hex-2(*trans*)-enal at the first site of contact and in the liver where higher levels of DNA adducts were observed than in other tissues investigated. Therefore, the new data provided by the Industry do not rule out the genotoxicity concern for the substances of subgroup 1.1.1.

For both 2-dodecenal and 2-nonenal tested through micronucleus assays in mouse bone marrow PCE (Honarvar, 2007b; Honarvar, 2008) there was no direct confirmation that the bone marrow was exposed, as no toxicokinetic measures of the test substance in plasma were made.

Under these conditions, the Panel confirms, the need for an *in vivo* Comet assay performed in duodenum and liver for hex-2(*trans*)-enal [FL-no: 05.073]. For the two other representative substances of subgroup 1.1.1 (nona-2(*trans*),6(*cis*)-dienal [FL-no: 05.058] and oct-2-enal [FL-no: 05.060]), a combined *in vivo* Comet assay and micronucleus assay would be required. For the latter, evidence of target tissue exposure should be provided.

SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 200REV1

Table 3: Specification Summary of the Substances in the Present Group Evaluation

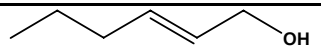
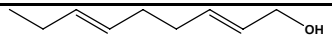
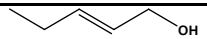
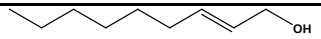
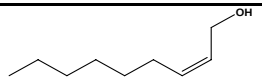
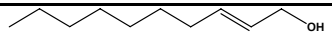
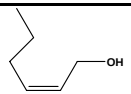
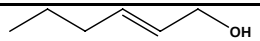
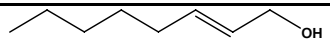
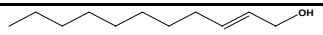
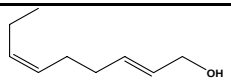
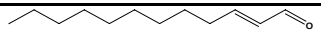
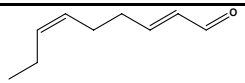
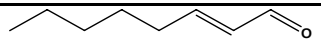
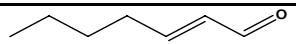
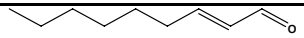
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
02.020 1354	Hex-2-en-1-ol		2562 69 2305-21-7	Liquid $C_6H_{12}O$ 100.16	Very slightly soluble Soluble	158-160 IR 95 %	1.437-1.442 0.836-0.841
02.049 1184	Nona-2,6-dien-1-ol		2780 589 7786-44-9	Liquid $C_9H_{16}O$ 140.23	Insoluble Soluble	196 IR NMR MS 95 %	1.463-1.465 0.860-0.880
02.050 1793	Pent-2-en-1-ol		665 20273-24-9	Liquid $C_5H_{10}O$ 86.13	Freely soluble	141 MS 95 %	1.427-1.433 0.844-0.850
02.090 1365	Non-2(trans)-en-1-ol		3379 10292 31502-14-4	Liquid $C_9H_{18}O$ 142.23	Insoluble Soluble	105 (16 hPa) IR 95 %	1.444-1.448 0.835-0.845
02.112 1369	Non-2(cis)-en-1-ol		3720 10292 41453-56-9	Liquid $C_9H_{18}O$ 142.23	Slightly soluble Soluble	96 (13 hPa) NMR 96 %	1.447-1.453 0.841-0.847
02.137 1794	Dec-2-en-1-ol		11750 22104-80-9	Liquid $C_{10}H_{20}O$ 156.27	Freely soluble	117 (19 hPa) MS 95 %	1.446-1.452 0.842-0.848
02.156 1374	Hex-2(cis)-en-1-ol		3924 69 928-94-9	Liquid $C_6H_{12}O$ 100.16	Insoluble Soluble	65 (0.7 hPa) NMR 92 %	1.437-1.445 0.845-0.853
02.157	Hex-2(trans)-en-1-ol		2562 69 2305-21-7				

Table 3: Specification Summary of the Substances in the Present Group Evaluation

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
02.192	Oct-2-en-1-ol		3887 11804 22104-78-5	Liquid $C_8H_{16}O$ 128	Insoluble Soluble	88 (hPa) MS 96 %	1.4371-1.4571 0.8384-0.8584
02.210 1384	Undec-2-en-1-ol		4068 37617-03-1	Liquid $C_{11}H_{22}O$ 170.30	Insoluble Soluble	100-102 (3 hPa) IR 95 %	1.447-1.453 0.838-0.848
02.231	tr-2, cis-6-Nonadien-1-ol		2780 589 28069-72-9	Liquid $C_9H_{16}O$ 140.23	Insoluble Soluble	196 MS 95 %	1.463-1.465 0.860-0.880
05.037 1350	2-Dodecenal		2402 124 4826-62-4	Liquid $C_{12}H_{22}O$ 182.31	Practically insoluble or insoluble Freely soluble	272 IR 93 %	1.452-1.458 0.839-0.849
05.058 1186	Nona-2(trans),6(cis)-dienal		3377 659 557-48-2	Liquid $C_9H_{14}O$ 138.21	Insoluble Soluble	94 IR 92 %	1.470-1.475 0.850-0.870
05.060 1363	Oct-2-enal		3215 663 2363-89-5	Liquid $C_8H_{14}O$ 126.20	Slightly soluble Soluble	84-86 (25 hPa) IR 92 %	1.449-1.455 0.835-0.845
05.070 1360	2-Heptenal		3165 730 2463-63-0	Liquid $C_7H_{12}O$ 112.17	Practically insoluble or insoluble Freely soluble	166 IR MS 97 %	1.428-1.434 0857-0.863
05.072 1362	trans-2-Nonenal		3213 733 18829-56-6	Liquid $C_9H_{16}O$ 140.22	Practically insoluble or insoluble Freely soluble	90 (1,2T) 1.333 IR MS 92 %	1.454-1.460 0.855-0.865

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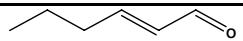
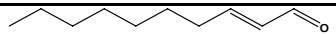
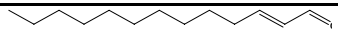
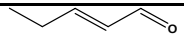
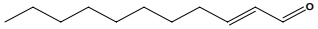
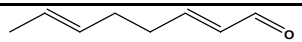
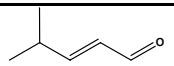
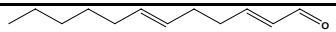
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
05.073 1353	Hex-2(trans)-enal		2560 748 6728-26-3	Liquid $C_6H_{10}O$ 98.14	Very slightly soluble Freely soluble	47 (1.7T) 2.266 NMR MS 92 %	1.443-1.449 0.841-0.848
05.076 1349	Dec-2-enal		2366 2009 3913-71-1	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Soluble	229 IR 92 %	1.452-1.458 0.836-0.846
05.078 1359	Tridec-2-enal		3082 2011 7774-82-5	Liquid $C_{13}H_{24}O$ 196.33	Insoluble Soluble	115-118 (13hPa) IR 92 %	1.455-1.461 0.842-0.862
05.102 1364	Pent-2-enal		3218 10375 764-39-6	Liquid C_5H_8O 84.11	Insoluble Soluble	124 NMR 98 %	1.440-1.447 (21°) 0.850-0.856 (21°)
05.109 1366	2-Undecenal		3423 11827 2463-77-6	Liquid $C_{11}H_{20}O$ 168.27	Insoluble Soluble	115 (13 hPa) NMR 98 %	1.452-1.459 0.837-0.847
05.111 1182	Octa-2(trans),6(trans)-dienal		3466 10371 56767-18-1	Liquid $C_8H_{12}O$ 124.19	Insoluble Soluble	97-99 (5 hPa) IR NMR 96 %	1.469-1.475 0.835-0.841
05.114 1208	4-Methylpent-2-enal		3510 10364 5362-56-1	Liquid $C_6H_{10}O$ 98.14	Slightly soluble Soluble	126-130 IR NMR 97 %	1.435-1.445 0.858-0.866
05.120 1197	Dodeca-2,6-dienal		3637 21662-13-5	Liquid $C_{12}H_{20}O$ 180.28	Insoluble Soluble	130 (7 hPa) NMR 97.5 %	1.425-1.431 0.987-0.993

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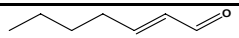
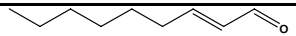
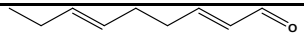
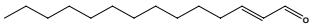
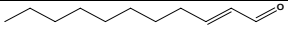
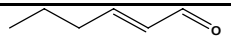
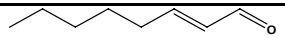
05.144	Dodec-2(trans)-enal		2402 20407-84-5				
05.150 1360	Hept-2(trans)-enal		3165 730 18829-55-5	Liquid $C_7H_{12}O$ 112.17	Insoluble Soluble	165-167 IR 97 %	1.428-1.434 0.857-0.863
05.171 1362	Non-2-enal	 (E)-isomer shown	3213 733 2463-53-8	Liquid $C_9H_{16}O$ 140.22	Insoluble Soluble	88-90 (16 hPa) IR 92 %	1.454-1.460 0.855-0.865
05.172 1187	Nona-2(trans),6(trans)-dienal		3766 17587-33-6	Liquid $C_9H_{14}O$ 138.21	Insoluble Soluble	88 (14 hPa) NMR 97 %	1.439-1.445 0.856-0.864
05.179 1803	Tetradec-2-enal		4209 51534-36-2	Solid $C_{14}H_{26}O$ 210.36	Freely soluble	88 (0.3 hPa) 35 MS 95 %	1.455-1.562 n.a.
05.184	Undec-2(trans)-enal		3423 11827 53448-07-0	Liquid $C_{11}H_{20}O$ 168.27	Insoluble Soluble	115 (1.3 hPa) MS 98 %	1.452-1.459 0.837-0.847
05.189	2-Hexenal		 748 505-57-7				
05.190	trans-2-Octenal		3215 2548-87-0	Liquid $C_8H_{14}O$ 126.2	Soluble Soluble	96 (2.5 hPa) MS 92 %	1.449-1.455 0.835-0.845

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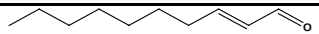
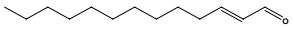
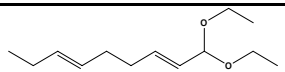
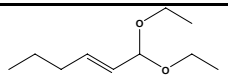
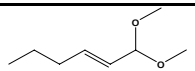
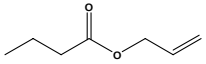
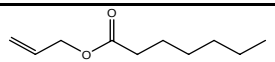
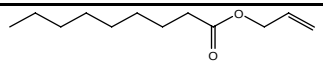
05.191	trans-2-Decenal		2366 3913-81-3				
05.195	trans-2-Tridecenal		3082 7069-41-2	Liquid $C_{13}H_{24}O$ 196.33	Insoluble Soluble	117 (1.3 hPa) MS 92 %	1.455-1.462 0.842-0.862
06.025 946	1,1-Diethoxynona-2,6-diene		3378 660 67674-36-6	Liquid $C_{13}H_{24}O_2$ 212.33	Insoluble Miscible	125 (5 hPa) IR 90 %	1.441-1.448 0.860-0.868
06.031 1383	1,1-Diethoxyhex-2-ene		4047 2135 54306-00-2	Liquid $C_{10}H_{20}O_2$ 172.27	Practically insoluble or insoluble Freely soluble	66 (8T) 10.6657 MS 95 %	1.418-1.426 0.843-0.849
06.072 1728	1,1-Dimethoxyhex-2(trans)-ene		 18318-83-7	Liquid $C_8H_{16}O_2$ 144.21	 Freely soluble	158 NMR 95 %	1.420-1.424 0.867-0.871
09.054 2	Allyl butyrate		2021 280 2051-78-7	Liquid $C_7H_{12}O_2$ 128.17	Insoluble Soluble	44-45 (20 hPa) IR 98 %	1.412 - 1.418 0.897 - 0.902
09.097 4	Allyl heptanoate		2031 369 142-19-8	Liquid $C_{10}H_{18}O_2$ 170.25	 Freely soluble	210 IR 97 %	1.426 - 1.430 0.880 - 0.885
09.109 6	Allyl nonanoate		2036 390 7493-72-3	Liquid $C_{12}H_{22}O_2$ 198.31	Insoluble Soluble	241-242 IR 96.5 %	1.430 - 1.436 0.872 - 0.880

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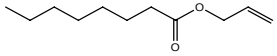
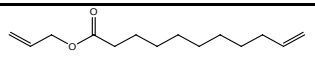
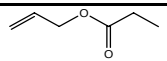
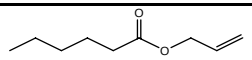
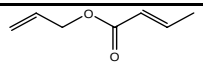
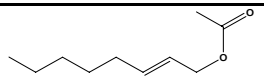
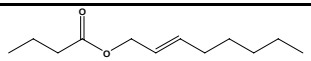
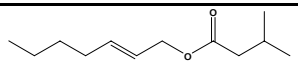
09.119 5	Allyl octanoate		2037 400 4230-97-1	Liquid $C_{11}H_{20}O_2$ 184.28	Insoluble Soluble	222 IR 97 %	1.432 - 1.434 0.872 - 0.880
09.146 9	Allyl undec-10-enoate		2044 441 7493-76-7	Liquid $C_{14}H_{24}O_2$ 224.34	Insoluble Soluble	180 (39 hPa) IR 98 %	1.448 at 30° 0.8802 at 30°
09.233 1	Allyl propionate		2040 2094 2408-20-0	Liquid $C_6H_{12}O_2$ 114.15		122-123 IR 99 %	1.4105 0.914 at 20°
09.244 3	Allyl hexanoate		2032 2181 123-68-2	Liquid $C_9H_{16}O_2$ 156.22	Insoluble 1 ml in 6 ml 70% ethanol	185 IR 98 %	1.422 - 1.426 0.884 - 0.890
09.247	Allyl crotonate		4072 2222 20474-93-5	Liquid $C_7H_{10}O_2$ 126.15	Freely soluble	146 MS 95 %	 0.932-0.937
09.276 1367	Oct-2-enyl acetate		3516 11906 3913-80-2	 170.25			
09.277 1368	Oct-2(trans)-enyl butyrate		3517 11907 84642-60-4	Liquid $C_{12}H_{22}O_2$ 198.30	Insoluble Soluble	112-113 (10hPa) IR NMR MS 96 %	1.433-1.439 0.890-0.896
09.303 1799	Hept-2-enyl isovalerate	 (E)-isomer shown	4126 10664	Liquid $C_{12}H_{22}O_2$ 198.30	Freely soluble	263 NMR 95 %	 0868-0.873

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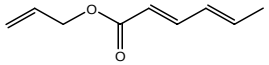
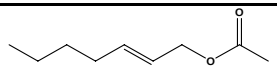
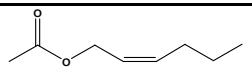
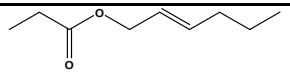
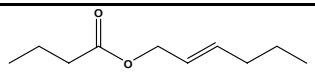
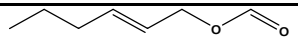
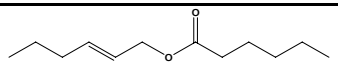
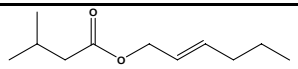
09.312 8	Allyl hexa-2,4-dienoate		2041 2182 7493-75-6	Liquid $C_9H_{12}O_2$ 152.19	 Soluble	67 IR 99 %	1.506 0.945 -0.947
09.385 1798	Hept-2-enyl acetate	 (E)-isomer shown	4125 10661 16939-73-4	Liquid $C_9H_{16}O_2$ 156.22	 Freely soluble	193 MS 95 %	1.428-1.434 0.889-0.895
09.394 1355	Hex-2(trans)-enyl acetate		2564 643 2497-18-9	Liquid $C_8H_{14}O_2$ 142.20	Very slightly soluble Soluble	165-166 IR 90 %	1.424-1.430 0.890-0.897
09.395 1378	Hex-2(trans)-enyl propionate		3932 11830 53398-80-4	Liquid $C_9H_{16}O_2$ 156.23	Insoluble Soluble	91 (26 hPa) NMR 95 %	1.426-1.433 0.885-0.895
09.396 1375	Hex-2-enyl butyrate		3926 53398-83-7				
09.397 1376	Hex-2-enyl formate		3927 11858 53398-78-0				
09.398 1381	Hex-2-enyl hexanoate		3983 53398-86-0				
09.399 1377	Hex-2-enyl isovalerate		3930 35154-45-1	Liquid $C_{11}H_{20}O_2$ 184.28	Insoluble Soluble	105 (26 hPa) NMR 96 %	1.425-1.435 0.875-0.885

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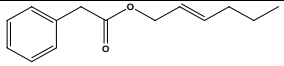
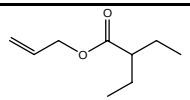
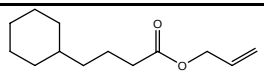
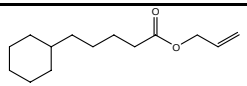
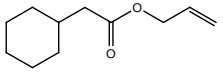
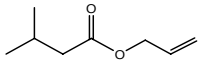
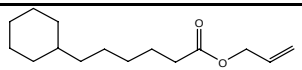
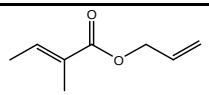
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
09.400	Hex-2-enyl phenylacetate		 68133-78-8	Solid $C_{14}H_{18}O_2$ 218.29	Practically insoluble or insoluble Freely soluble	336 37 NMR 95 %	n.a. n.a.
09.410 11	Allyl 2-ethylbutyrate		2029 281 7493-69-8	Liquid $C_9H_{16}O_2$ 156.23	Insoluble Soluble	165-167 IR 99 %	1.422 - 1.427 0.882 - 0.887
09.411 14	Allyl cyclohexanebutyrate		2024 283 7493-65-4	Liquid $C_{13}H_{22}O_2$ 210.31	Insoluble Soluble	104 (1 hPa) NMR 98 %	1.4608 at 20.5° 0.943-0.949
09.469 15	Allyl cyclohexanevalerate		2027 474 7493-68-7	Liquid $C_{14}H_{24}O_2$ 224.34	insoluble Soluble	119 (1 hPa) IR 98 %	1.4605 at 22° 0.942-0.947
09.482 12	Allyl cyclohexaneacetate		2023 2070 4728-82-9	Liquid $C_{11}H_{18}O_2$ 182.26	 Soluble	60 (1 hPa) NMR 96 %	1.455 - 1.499 0.945 - 0.965
09.489 7	Allyl isovalerate		2045 2098 2835-39-4	Liquid $C_8H_{14}O_2$ 142.20	Insoluble Freely soluble	155 IR 98 %	1.413-1.418 0.879 - 0.884
09.492 16	Allyl cyclohexanehexanoate		2025 2180 7493-66-5	Liquid $C_{14}H_{28}O_2$ 238.37	Insoluble Soluble	128 (2 hPa) NMR 98 %	1.462 0.941-0.947
09.493 10	Allyl 2-methylcrotonate		2043 2183 7493-71-2	Liquid $C_8H_{12}O_2$ 140.18	Slightly soluble	153 IR 98 %	1.451 - 1.454 0.939 - 0.943

Table 3: Specification Summary of the Substances in the Present Group Evaluation

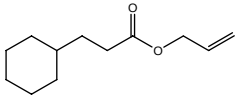
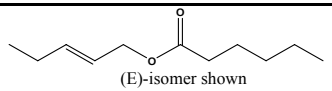
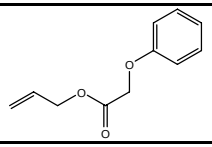
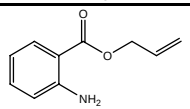
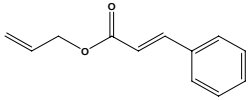
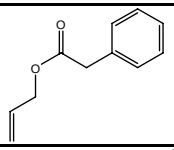
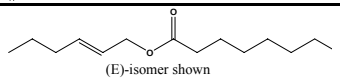
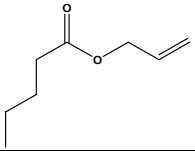
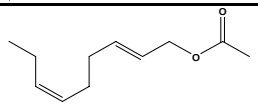
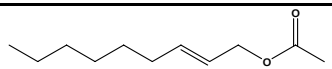
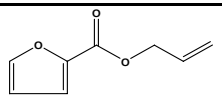
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]
09.498 13	Allyl cyclohexanepropionate		2026 2223 2705-87-5	Liquid $C_{12}H_{20}O_2$ 196.29	Insoluble 1 ml in 4 ml 80% ethanol	91 (1 hPa) IR 98 %	1.457 - 1.462 0.945 - 0.950
09.678 1795	Pent-2-enyl hexanoate	 (E)-isomer shown	4191 74298-89-8	Liquid $C_{11}H_{20}O_2$ 184.28	Freely soluble	241 MS 95 %	1.425-1.435 0.885-0.895
09.701 18	Allyl phenoxyacetate		2038 228 7493-74-5	Liquid $C_{11}H_{12}O_3$ 192.22		100-102 (1 hPa) IR 97.5 %	1.512 - 1.519 1.00 - 1.11
09.719 20	Allyl anthranilate		2020 254 7493-63-2	Liquid $C_{10}H_{11}O_2N$ 177.21	Almost insoluble	105 (3 hPa) IR 98 %	1.569-1.577 1.12
09.741 19	Allyl cinnamate		2022 334 1866-31-5	Liquid $C_{12}H_{12}O_2$ 188.22	Insoluble Miscible	286 IR 97 %	1.562-1.569 1.050-1.056
09.790 17	Allyl phenylacetate		2039 2162 1797-74-6	Liquid $C_{11}H_{12}O_2$ 176.22		89-93 (4 hPa) IR 99 %	1.5122 at 13.5° 1.033-1.041
09.841 1796	2-Hexenyl octanoate	 (E)-isomer shown	4135 85554-72-9	Liquid $C_{14}H_{26}O_2$ 226.36	Freely soluble	309 MS 95 %	

Table 3: Specification Summary of the Substances in the Present Group Evaluation

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [(a)] Solubility in ethanol [(b)]	Boiling point, °C [(c)] Melting point, °C ID test Assay minimum	Refrac. Index [(d)] Spec.gravity [(e)]
09.866	Allyl valerate		4074	Liquid $C_8H_{14}O_2$ 142.20	Freely soluble	58 (16 hPa) MS 95 %	0.999-1.005
09.947 1188	(E,Z)-2,6-Nonadienyl acetate		3952 68555-65-7	Liquid $C_{11}H_{18}O_2$ 182.26	Sparingly soluble Soluble	231 IR NMR MS 95 %	1.448-1.458 0.905-0.907
09.948	(2E)-2-Nonenyl acetate		4552 30418-89-4	Liquid $C_{11}H_{20}O_2$ 184.79	Sparingly soluble Very soluble	228 IR NMR MS 98 %	1.4325-1.4425 0.874-0.894
13.004 21	Allyl 2-furoate		2030 360 4208-49-5	Liquid $C_8H_8O_3$ 152.15		206-209 IR 98 %	1.4945 1.181 (23°)

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

SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE

Table 4: Summary of Safety Evaluation of the JECFA Substances in the Present Group

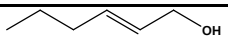
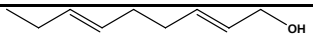
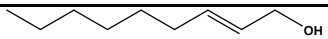
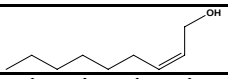
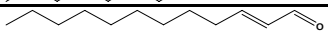
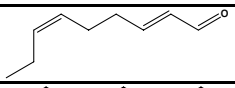
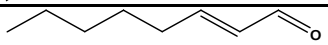
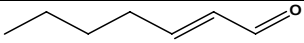
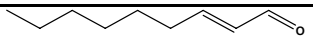
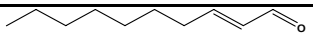
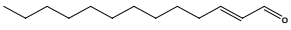
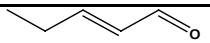
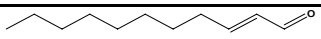
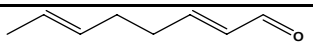
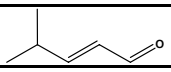
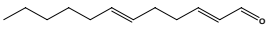
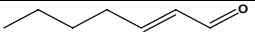
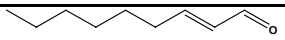
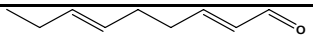
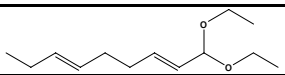
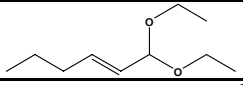
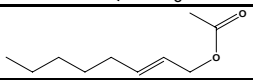
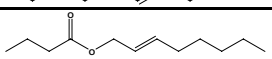
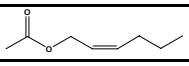
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/*capita*/day)	Class [b] Evaluation procedure path [c]	JECFA Outcome on the named compound (d) or (e)
02.020 1354	Hex-2-en-1-ol		340 291	Class I A3: Intake below threshold	(d)
02.049 1184	Nona-2,6-dien-1-ol		1.9 1	Class I A3: Intake below threshold	(d)
02.090 1365	Non-2(trans)-en-1-ol		0.12 0.03	Class I A3: Intake below threshold	(d)
02.112 1369	Non-2(cis)-en-1-ol		0.065 2	Class I A3: Intake below threshold	(d)
05.037 1350	2-Dodecenal		0.12 2	Class I A3: Intake below threshold	(d)
05.058 1186	Nona-2(trans),6(cis)-dienal		6.1 24	Class I A3: Intake below threshold	(d)
05.060 1363	Oct-2-enal		3.3 0.9	Class I A3: Intake below threshold	(d)
05.070 1360	2-Heptenal		5.4	Class I A3: Intake below threshold	(d)
05.072 1362	trans-2-Nonenal		0.12	Class I A3: Intake below threshold	(d)
05.076 1349	Dec-2-enal		2.6 6	Class I A3: Intake below threshold	(d)
05.078 1359	Tridec-2-enal		0.49 0.7	Class I A3: Intake below threshold	(d)
05.102 1364	Pent-2-enal		0.67 0.1	Class I A3: Intake below threshold	(d)
05.109 1366	2-Undecenal		0.33 0.4	Class I A3: Intake below threshold	(d)
05.111 1182	Octa-2(trans),6(trans)-dienal		0.12 0.007	Class I A3: Intake below threshold	(d)
05.114 1208	4-Methylpent-2-enal		0.24 0.2	Class I A3: Intake below threshold	(d)
05.120 1197	Dodeca-2,6-dienal		0.44 0.009	Class I A3: Intake below threshold	(d)
05.150 1360	Hept-2(trans)-enal		5.1 30	Class I A3: Intake below threshold	(d)
05.171 1362	Non-2-enal	 (E)-isomer shown	1.7 0.4	Class I A3: Intake below threshold	(d)
05.172 1187	Nona-2(trans),6(trans)-dienal		ND 0.007	Class I A3: Intake below threshold	(d)
06.025 946	1,1-Diethoxynona-2,6-diene		0.037 0.01	Class I A3: Intake below threshold	(d)
06.031 1383	1,1-Diethoxyhex-2-ene		0.24	Class I A3: Intake below threshold	(d)
09.276 1367	Oct-2-enyl acetate		0.18 0.7	Class I A3: Intake below threshold	(d)
09.277 1368	Oct-2(trans)-enyl butyrate		0.26 0.7	Class I A3: Intake below threshold	(d)
09.394 1355	Hex-2(trans)-enyl acetate		170 56	Class I A3: Intake below threshold	(d)

Table 4: Summary of Safety Evaluation of the JECFA Substances in the Present Group

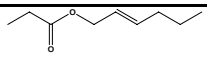
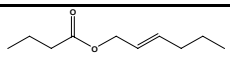
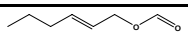
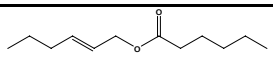
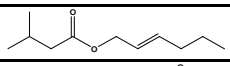
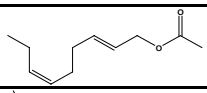
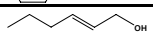
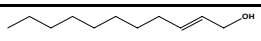
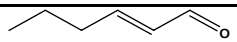
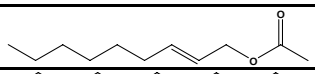
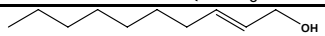
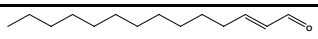
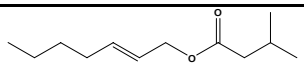
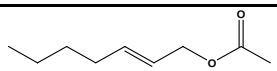
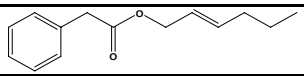
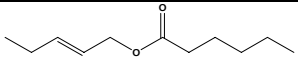
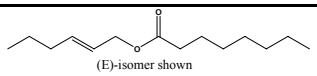
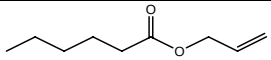
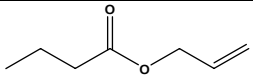
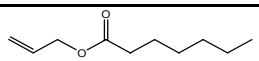
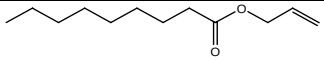
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/*capita*/day)	Class [b] Evaluation procedure path [c]	JECFA Outcome on the named compound [d] or [e]
09.395 1378	Hex-2(trans)-enyl propionate		ND 4	Class I A3: Intake below threshold	(d)
09.396 1375	Hex-2-enyl butyrate		ND 4	Class I A3: Intake below threshold	(d)
09.397 1376	Hex-2-enyl formate		ND 7	Class I A3: Intake below threshold	(d)
09.398 1381	Hex-2-enyl hexanoate		ND 0.09	Class I A3: Intake below threshold	(d)
09.399 1377	Hex-2-enyl isovalerate		ND 4	Class I A3: Intake below threshold	(d)
09.947 1188	(E,Z)-2,6-Nonadienyl acetate		1.2	Class I A3: Intake below threshold	(d)
02.156 1374	Hex-2(cis)-en-1-ol		ND 10	Class I No evaluation	
02.157	Hex-2(trans)-en-1-ol		340 291	Class I No evaluation	
02.210 1384	Undec-2-en-1-ol		ND 1	Class I No evaluation	
05.189	2-Hexenal		675 409	Class I No evaluation	
09.948	(2E)-2-Nonenyl acetate		1.2	Class I No evaluation	
02.137 1794	Dec-2-en-1-ol		0.12	Class I No evaluation	
05.179 1803	Tetradec-2-enal		0.061	Class I No evaluation	
09.303 1799	Hept-2-enyl isovalerate	 (E)-isomer shown	0.0012	Class I No evaluation	
09.385 1798	Hept-2-enyl acetate	 (E)-isomer shown	0.0061	Class I No evaluation	
09.400	Hex-2-enyl phenylacetate		0.012	Class I No evaluation	
09.678 1795	Pent-2-enyl hexanoate	 (E)-isomer shown	0.4	Class I No evaluation	
09.841 1796	2-Hexenyl octanoate	 (E)-isomer shown	0.012	Class I No evaluation	
09.244 3	Allyl hexanoate		2600 820	Class II B3: Intake above threshold	Data must be available e
09.054 2	Allyl butyrate		11 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.097 4	Allyl heptanoate		130 28	Class II A3: Intake above threshold, A4: Endogenous	(d)
09.109 6	Allyl nonanoate		<0.01 0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)

Table 4: Summary of Safety Evaluation of the JECFA Substances in the Present Group

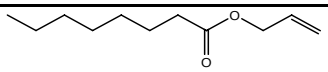
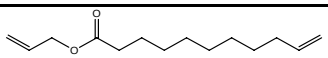
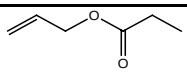
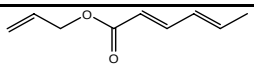
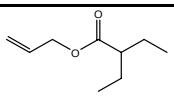
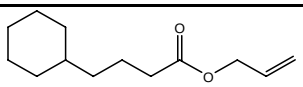
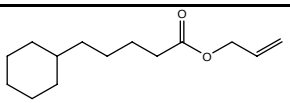
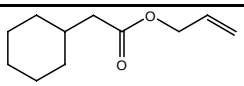
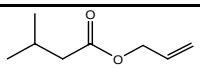
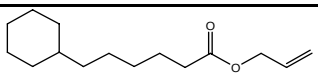
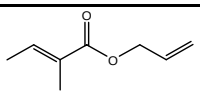
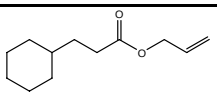
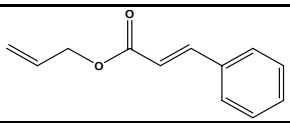
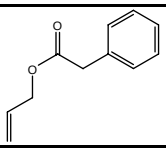
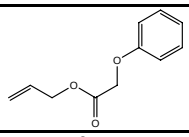
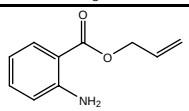
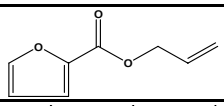
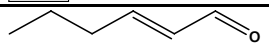
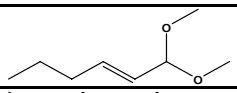
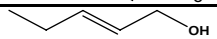
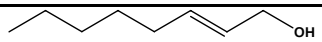
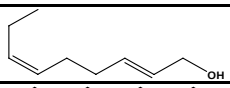
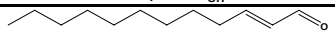
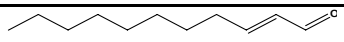
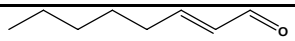
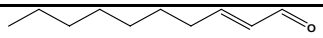
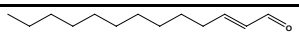
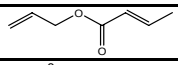
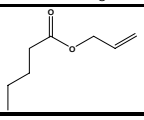
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/*capita*/day)	Class [b] Evaluation procedure path [c]	JECFA Outcome on the named compound [d] or [e]
09.119 5	Allyl octanoate		45 1.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.146 9	Allyl undec-10-enoate		<0.01 <0.01	Class II A3: Intake above threshold, A4: Endogenous	(d)
09.233 1	Allyl propionate		7.9 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.312 8	Allyl hexa-2,4-dienoate		<0.01 <0.01	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required
09.410 11	Allyl 2-ethylbutyrate		0.26 0.02	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.411 14	Allyl cyclohexanebutyrate		0.14 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.469 15	Allyl cyclohexanevalerate		0.14 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.482 12	Allyl cyclohexaneacetate		<0.01 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.489 7	Allyl isovalerate		0.38 0.19	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.492 16	Allyl cyclohexanehexanoate		0.36 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.493 10	Allyl 2-methylcrotonate		1.3 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.498 13	Allyl cyclohexanepropionate		220 110	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.741 19	Allyl cinnamate		4.6 0.28	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.790 17	Allyl phenylacetate		6.5 <0.01	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	(d)

Table 4: Summary of Safety Evaluation of the JECFA Substances in the Present Group

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	JECFA Outcome on the named compound [(d) or (e)]
09.701 18	Allyl phenoxyacetate		30 2.5	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
09.719 20	Allyl anthranilate		0.12 0.09	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	(d)
13.004 21	Allyl 2-furoate		0.12 <0.01	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required
05.073 1353	Hex-2(trans)-enal		670	A3: Intake below threshold	(d)
06.072 1728	1,1-Dimethoxyhex-2(trans)-ene		0.12	A3: Intake below threshold	(d)
02.050 1793	Pent-2-en-1-ol		0.57	A3: Intake below threshold	(d)
02.192	Oct-2-en-1-ol		1800	No evaluation	Not evaluated by the JECFA
02.231	tr-2,cis-6-Nonadien-1-ol		1.0	No evaluation	Not evaluated by the JECFA
05.144	Dodec-2(trans)-enal			No evaluation	Not evaluated by the JECFA
05.184	Undec-2(trans)-enal		0.34	No evaluation	Not evaluated by the JECFA
05.190	trans-2-Octenal		0.89	No evaluation	Not evaluated by the JECFA
05.191	trans-2-Decenal			No evaluation	Not evaluated by the JECFA
05.195	trans-2-Tridecenal		0.11	No evaluation	Not evaluated by the JECFA
09.247	Allyl crotonate		0.41	No evaluation	Not evaluated by the JECFA
09.866	Allyl valerate		0.012	No evaluation	Not evaluated by the JECFA

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

(Q)SAR PREDICTIONS ON MUTAGENICITY FOR ALDEHYDES FROM SUBGROUP 1.1.1

Table 5: QSAR Predictions on Mutagenicity for 25 Aldehydes from Subgroup 1.1.1

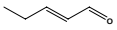
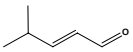
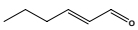
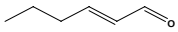
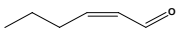
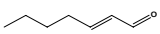
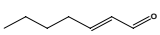
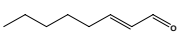
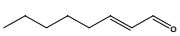
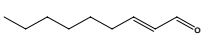
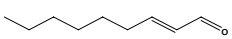
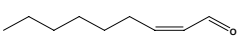
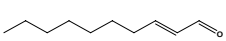
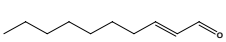
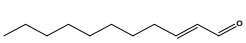
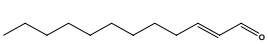
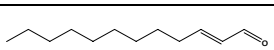
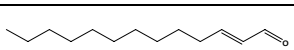
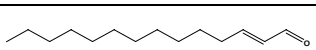
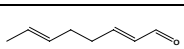
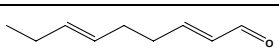
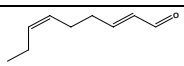
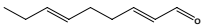
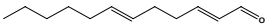
FL-no JECFA-no	EU Register name	Structural formula [a]	ISS Local Model Ames Test TA100 [b]	MultiCASE Ames test [c]	MultiCASE Mouse lymphoma test [d]	Mul Chro aberra C
05.176	Prop-2-enal		POS	POS	OD	
05.102 1364	Pent-2-enal		POS	POS	OD	
05.114 1208	4-Methylpent-2-enal		POS	NEG	OD	
05.189 1353	2-Hexenal		POS	POS	OD	
05.073	Hex-2(trans)-enal		POS	POS	OD	
Not in Register	Hex-2(cis)-en-1-al		POS	POS	OD	
05.150 1360	Hept-2(trans)-enal		POS	POS	OD	
05.070	2-Heptenal		POS	POS	OD	
05.060 1363	Oct-2-enal		POS	EQU	OD	
05.190	trans-2-Octenal		POS	EQU	OD	
05.171 1362	Non-2-enal		POS	EQU	OD	
05.072	trans-2-Nonenal		POS	EQU	OD	
Not in Register	Non-2(cis)-en-1-al		POS	EQU	OD	
05.076 1349	Dec-2-enal		POS	EQU	OD	
05.191	trans-2-Decenal		POS	EQU	OD	
05.109 1366	2-Undecenal		POS	EQU	OD	
05.144	Dodec-2(trans)-enal		POS	EQU	OD	
05.037 1350	2-Dodecenal		POS	EQU	OD	
05.078 1359	Tridec-2-enal		POS	EQU	OD	
05.179	Tetradec-2-enal		POS	EQU	OD	
05.111 1182	Octa-2(trans),6(trans)-dienal		NEG	EQU	OD	
Not in Register	Nona-2,6-dien-1-al		NEG	NEG	OD	
05.058 1186	Nona-2(trans),6(cis)-dienal		NEG	NEG	OD	

Table 5: QSAR Predictions on Mutagenicity for 25 Aldehydes from Subgroup 1.1.1

FL-no JECFA-no	EU Register name	Structural formula [(a)]	ISS Local Model Ames Test TA100 [(b)]	MultiCASE Ames test [(c)]	MultiCASE Mouse lymphoma test [(d)]	Mul Chro aberra Cl
05.172 1187	Nona-2(trans),6(trans)-dienal		NEG	NEG	OD	
05.120 1197	Dodeca-2,6-dienal		NEG	EQU	OD	

(a): Structure subgroup.
(b): Local model on aldehydes and ketones, Ames TA100. (NEG: Negative; POS: Positive; OD: out of domain).
(c): MultiCase Ames test (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(d): MultiCase Mouse Lymphona test (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(e): MultiCase Chromosomal aberration in CHO (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
(f): MultiCase Chromosomal aberration in CHL (OD: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).

GENOTOXICITY DATA

Table 6: Genotoxicity Data (*in vitro*) Considered by the Panel

Register name [FL-no]	End-point	Test system	Concentration	Results	Reference	Remark
Nona-2(*trans*),6(*cis*)-dienal [05.058]	Reverse mutation	*S. typhimurium* TA100	0.01 – 0.1 µl/plate (8.6 – 86 µg/plate)[a] [4,1]	Negative	Eder et al., 1992	Valid. Standard 30-min [a] Calcula 0.870g/
	Reverse mutation	*S. typhimurium* TA100	0.005 – 0.15 µl/plate (4.3 – 129 µg/plate)[a] [4,1] 0.005 – 0.20 µl/plate (4.3 – 172 µg/plate)[a] [4,2]	Negative	Eder et al., 1992	Valid. Three-f 90-min [a] Calcula 0.870g/
	SOS chromotest	*E. coli* PQ37 and PQ243	5 – 80 nmol (0.69 – 11 µg/l)	Negative	Eder et al., 1992	Valid.
	Sister chromatid exchange	Human lymphoblastoid Namalva cell line	0 – 40 µM (0 – 5.5 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Sister chromatid exchange	Primary human blood lymphocytes	0 – 50 µM (0 – 6.9 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Structural chromosomal aberration test	Human lymphoblastoid Namalva cell line	0 – 40 µM (0 – 5.5 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Structural chromosomal aberration test	Primary human blood lymphocytes	0 – 40 µM (0 – 5.5 µg/ml) [1]	Equivocal	Dittberner et al., 1995	Valid.
	Numerical chromosomal aberration test	Primary human blood lymphocytes	0 - 40 µM (0 - 5.5 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Micronucleus formation	Primary human blood lymphocytes	0 – 50 µM (0 – 6.9 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Micronucleus formation	Human lymphoblastoid Namalva cell line	0 – 50 µM (0 – 6.9 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
Hex-2(*trans*)-enal [05.073]	Reverse Mutation	*S. typhimurium* TA98, TA100, and TA104	Not reported [4,5]	Positive	Kato et al., 1989	Validity Accord "suspect however Liquid p
	Reverse Mutation	*S. typhimurium* TA100	0.05 - 0.35 µl/plate [4,1] 0.15 – 0.5 µl/plate [4,2]	Negative	Eder et al., 1992	Valid. Standard 30-min
	Reverse Mutation	*S. typhimurium* TA100	0.01 - 0.15 µl/plate [4,1] 0.1 – 0.4 µl/plate [4,2]	Positive	Eder et al., 1992	Valid. Three-f 90-min
	SOS Chromotest	*E. coli* PQ37 and PQ243	70 - 435 nmol (6.9 - 42.7 µg)[a]	Negative	Eder et al., 1992	Valid. Cytotox tested. [a] Calcul hexenal

Register name [FL-no]	End-point	Test system	Concentration	Results	Reference	Remark
	Mutation	*E. coli* WP2uvrA/pKM 101	Not reported [5]	Positive	Kato et al., 1989	Validity Accordi "suspect however Liquid p
	Micronucleus Induction	Human blood lymphocytes	5 - 250 µM (0.5 - 24.5 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Micronucleus Induction	Lymphoblastoid Namalva cells	5 - 250 µM (0.5 - 24.5 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Chromosomal Aberration	Human blood lymphocytes	5 - 250 µM (0.5 - 24.5 µg/ml) [1]	Negative	Dittberner et al., 1995	Valid.
	Chromosomal Aberration	Lymphoblastoid Namalva cells	5 - 150 µM (0.5 - 14.7 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Sister Chromatid Exchange	Human blood lymphocytes	5 - 250 µM (0.5 - 24.5 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	Sister Chromatid Exchange	Lymphoblastoid Namalva cells	5 - 200 µM (0.5 - 19.6 µg/ml) [1]	Positive	Dittberner et al., 1995	Valid.
	DNA Repair	Rat hepatocytes	60 - 600 nmol/10^6 cells (5.9 - 58.9 µmol)[a]	Positive	Griffin and Segall, 1986	Valid Study de 482. UD concentr [a] Calcul hexenal
Pent-2-enal [05.102]	Reverse mutation	*S. typhimurium* TA100	0.075 – 0.5 µl/plate [4,1] 0.075 – 0.75 µl/plate [4,2]	Positive	Eder et al., 1992	Valid. Standard 30-min p
	Reverse mutation	*S. typhimurium* TA100	0.01 – 0.25 µl/plate [4,1] 0.1 – 0.4 µl/plate [4,2]	Positive	Eder et al., 1992	Valid. Three-fo 90-min p
	SOS chromotest	*E. coli* PQ37 and PQ243	60 – 435 nmol (5.0 – 36.7 µg)[a]	Negative	Eder et al., 1992	Valid. Cytotox tested. [a] Calcul pentenal
	Mutation induction TG resistance	Chinese hamster V79 cells	0.03, 0.10 or 0.30 mM (2.5, 8.4 or 25.2 µg/ml)[a] [1]	Positive	Canonero et al., 1990	Limited No data doses w dose ran number [a] Calcul pentenal
	Mutation induction Ouavaine resistance	Chinese hamster V79 cells	0.03, 0.10 or 0.30 mM (2.5, 8.4 or 25.2 µg/ml)[a] [1]	Negative	Canonero et al., 1990	Limited No data doses w dose ran [a] Calcul pentenal
	DNA single strand break	Mouse leukaemia cells L1210	400, 600 or 800 µmol (33.648, 50.472 or 67.296 µg)[a]	Positive	Eder et al., 1993	Limited Results the auth positive starting. highest [a] Calcul pentenal
2-Heptenal [05.070]	Reverse mutation	*S. typhimurium* TA104	Up to 0.9 µmol/plate[a] (101 µg/plate)[b] [4,1]	Negative	Marnett et al., 1985	Validity Results incubati [a] Maxim

Register name [FL-no]	End-point	Test system	Concentration	Results	Reference	Remark
						[b] Calcul heptenal
	Reverse mutation	*S. typhimurium* TA104	Up to 4.4 µmol/plate[a] (493.5 µg/plate)[b][4,1]	Negative	Marnett et al., 1985	Validity Results incubati glutathio [a] Maxim [b] Calcul heptenal
	Reverse mutation	*S. typhimurium* TA100	0.01 – 0.15 µl/plate [4,1] 0.075 – 0.3 µl/plate [4,2]	Negative	Eder et al., 1992	Valid. Standard min pre- in mutat these inc the spon
	Reverse mutation	*S. typhimurium* TA100	0.005 – 0.1 µl/plate [4,1] 0.025 – 0.3 µl/plate [4,2]	Negative	Eder et al., 1992	Valid. Three-fo used. 90 Dose-de frequenc were ne spontane
	SOS chromotest	E. coli PQ37 and PQ243	35 – 270 nmol (3.9 – 30.3 µg)[a]	Negative	Eder et al., 1992	Valid. Cytotox tested. [a] Calcul heptenal
	Mutation induction TG resistance	Chinese hamster V79 cells	0.01, 0.03 or 0.10 mM (1.1, 3.4 or 11.2 µg/ml)[a] [1]	Positive	Canonero et al., 1990	Limited No data doses w dose ran number were ob [a] Calcul heptenal
	Mutation induction Ouabaine resistance	Chinese hamster V79 cells	0.01, 0.03 or 0.10 mM (1.1, 3.4 or 11.2 µg/ml)[a] [1]	Negative	Canonero et al., 1990	Limited No data doses w dose ran [a] Calcul heptenal
	DNA single strand break	Mouse leukaemia L1210 cells	200, 400 or 500 µmol (22.434, 44.868 or 56.085 µg)[a]	Positive	Eder et al., 1993	Limited Results the auth non-toxi [a] Calcul heptenal
trans-2-Nonenal [05.072]	Micronucleus formation	Rat hepatocytes	0.1, 1, 10 or 100 µM (0.01, 0.1, 1.4 or 14.0 µg/ml)	Positive	Esterbauer et al., 1990	Limited Difficult expresse per mito the resul this stud al. (199
	Micronucleus formation	Rat hepatocytes	0.1, 10 or 100 µM (0.01, 1.4 or 14.0 µg/ml)	Equivocal	Eckl et al., 1993	Limited Cells we treatmen
	Chromosomal aberration	Rat hepatocytes	0.1, 1, 10 or 100 µM (0.01, 0.1, 1.4 or 14.0 µg/ml)	Negative	Esterbauer et al., 1990	Validity of chron

Register name [FL-no]	End-point	Test system	Concentration	Results	Reference	Remark
	Chromosomal aberration	Rat hepatocytes	0.1, 10 or 100 µM (0.01, 1.4 or 14.0 µg/ml)	Negative	Eckl et al., 1993	Validity of chron Cells we treatmer
	Sister chromatid exchange	Rat hepatocytes	0.1, 10 or 100 µM (0.01, 1.4 or 14.0 µg/ml)	Equivocal	Eckl et al., 1993	Limited Cells we treatmer
	DNA repair	Rat hepatocytes	60 – 600 nmol/10^6 cells (8.4 – 84.1 µg/plate)	Positive	Griffin and Segall, 1986	Valid. S Guideli highest concentr increase net grai
Non-2-enal [05.171]	Reverse mutation	*S. typhimurium* TA104	Up to 0.007 µmol/plate [a] (1.0 µg/plate) [4,1]	Negative	Marnett et al., 1985	Validity Results Liquid p [a] Maxim
	Mutation induction TG resistance	Chinese hamster V79 cells	0.003 or 0.01 mM (0.4 or 1.4 µg/ml) [1]	Positive	Canonero et al., 1990	Limited No data doses w dose ran number
	Mutation induction Ouabaine resistance	Chinese hamster V79 cells	0.003 or 0.01 mM (0.4 or 1.4 µg/ml) [1]	Negative	Canonero et al., 1990	Limited No data doses w dose ran
2-Hexenal [05.189]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	3 µmol/plate (294.4 µg/plate)[a] [5]	Negative	Florin et al., 1980	Insuffici OECD G spot test Isomeric given. [a] Calcul hexenal
	Reverse Mutation	*S. typhimurium* TA104	Up to 2 µmol/plate[a] (196.3 µg/plate)[b] [4,1]	Positive	Marnett et al., 1985	Validity Results composi pre-incu [a] Maxim [b] Calcul hexenal
	Reverse Mutation	*S. typhimurium* TA104	5 µmol/plate[a] (> 490.7 µg/plate)[b] [4,1]	Positive	Marnett et al., 1985	Validity Results Isomeric given. L used. Ac [a] Maxim [b] Calcul hexenal
	Reverse Mutation	*S. typhimurium* TA102	Up to 2 µmol/plate[a] (196.3 µg/plate)[b] [4,1]	Negative	Marnett et al., 1985	Validity Results composi pre-incu [a] Maxim [b] Calcul hexenal
	Mutation induction TG resistance	Chinese hamster V79 cells	0.03, 0.10 or 0.30 mM (2.9, 9.8 or 29.4 µg/ml)[a] [1]	Positive	Canonero et al., 1990	Limited No data doses w dose ran number

Register name [FL-no]	End-point	Test system	Concentration	Results	Reference	Remark
						[a] Calcul hexenal
	Mutation induction Ouabaine resistance	Chinese hamster V79 cells	0.03, 0.10 or 0.30 mM (2.9, 9.8 or 29.4 µg/ml)[a] [1]	Negative	Canonero et al., 1990	Limited No data doses w dose ran [a] Calcul hexenal
	DNA Single Strand Break	L1210 mouse leukemia cells	100, 250 or 500 µmol (9.814, 24.535 or 49.070µg)[a]	Positive	Eder et al., 1993	Limited Results the auth non-toxi the high of test s [a] Calcul hexenal
2-Octenal [05.060]	Bacterial Reverse Mutation	*S. typhimurium* TA104	Up to 0.8 µmol/plate[a] (101.0 µg/plate)[b] [4,1]	Negative	Marnett et al., 1985	Validity Results incubati [a] Maxim [b] Calcul octenal
		S. typhimurium TA104	Up to 4 µmol/plate[a] (504.8 µg/plate)[b] [4,1]	Negative	Marnett et al., 1985	Validity Results incubati Additio [a] Maxim [b] Calcul octenal
	Mutation Induction TG resistance Ouabain resistance	Chinese hamster V79 cells	0.01, 0.03 or 0.10 mM (1.3, 3.8 or 12.6 µg/ml) [1]	Positive (TG resistance: HPRT mutation) Negative (Ouabain resistance)	Canonero et al., 1990	Limited No data doses w dose ran The test activatio controls mutants
	DNA Single Strand Breaks	L1210 mouse leukemia cells	250, 350 µmol (44 mg/plate)	Positive	Eder et al., 1993	Limited Results the auth 350 µm

[1] Without S9 metabolic activation.
[2] With S9 metabolic activation.
[3] Plate incorporation method.
[4] Pre-incubation method.
[5] With and without S9 metabolic activation.

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

Table 7: Genotoxicity Data (*in vivo*) Considered by the Panel

Register name [FL-no]	End-point	Test system	Concentration	Results	Reference
Hex-2(trans)-enal [05.073]	Micronucleus Induction	Human buccal mucosa cells	10 mg/kg	Positive	Dittberner et al., 1997

Table 8: Additional Genotoxicity Data (*in vitro*) Considered by the Panel in FGE.200

Register name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Hex-2(trans)-enal [05.073]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	3 - 5000 µg/plate [3,5]	Negative	Sokolowski, 2007a	A moderate concentration – dependent increase in revertant colony number was observed in strain TA100, in the absence of S9-mix. Study design complies with OECD Guidelines 471
		S. typhimurium TA100	1 - 2500 µg/plate [4,5]	Positive[4,1]		
		S. typhimurium TA100	25- 200 µg/plate [4,5]	Positive[4,1]		
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	3- 5000 µg/plate [3,5]	Negative	Bhatia et al., 2010	Same test as Sokolowski, 2007a
		S. typhimurium TA100	1 - 2500 µg/plate [4,5]	Positive [4,1]		
		S. typhimurium TA100	25 -200 µg/plate [4,1]	Positive [4,1]		
2-Dodecenal [05.037]	Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537	3 - 5000 µg/plate [3,5]	Negative	Sokolowski, 2007b	Concentrations up to 5000 µg/plate were used in a pre-experiment test. Toxic effects as a reduction in the number of revertants were observed at the higher concentrations.
			0.1– 100 µg/plate [3,1] 1 - 1000 µg/plate [3,2]	Negative		
			0.3 - 1000 µg/plate [4,5]	Negative		
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537	1 - 1000 µg/plate [3,2] 0.1 - 100 µg/plate [3,1]	Negative	Bhatia et al., 2010	Same test as Sokolowski, 2007b

[1] Without S9 metabolic activation.
[2] With S9 metabolic activation.
[3] Plate incorporation method.
[4] Pre-incubation method.
[5] With and without S9 metabolic activation.

Table 9: Additional Genotoxicity Data (*in vivo*) Considered by the Panel in FGE.200

Register name [FL-no]	Test System *in vivo*	Test Object	Doses	Result	Reference	Comments
trans-2-Hexenal [05.073]	Micronucleus Assay	Mouse bone marrow polychromatic erythrocytes	250, 500 and 1000 mg/kg bw	Negative	Honarvar, 2007a	Study design complies with OECD Guideline 474.
	Unscheduled DNA Synthesis	Male rats hepatocytes	200 or 500 mg/kg bw	Negative	Durward, 2009	Purity and isomer were not specified. Study design complies with OECD Guideline 486.
	Micronucleus Assay	Transgenic Muta™Mouse (CD2-lacZ80/HazfBR) blood erythrocytes and reticulocytes	120, 235 and 350 mg/kg/day	Negative	Beevers, 2013	Mice were treated by gavage for 28 days. The dose of 350 mg/kg/day was identified as MTD.Deviations from OECD Guideline 474 were identified.
	Induction of *lacZ*⁻ mutation	Transgenic Muta™Mice (CD2-lacZ80/HazfBR) liver and duodenum	120, 235 and 350 mg/kg/day	Negative	Beevers, 2013	Mice were treated by gavage for 28 days. The dose of 350 mg/kg/day was identified as MTD. Liver and duodenum were analysed. Study design complies with OECD Guideline 488.
	Micronucleus Assay	Mouse bone marrow polychromatic erythrocytes	250, 500 and 1000 mg/kg bw	Negative	Bhatia et al., 2010	Same test as Honarvar, 2007a.
2-Nonenal [05.171]	Micronucleus Assay	Mouse bone marrow polychromatic erythrocytes	500, 1000 and 2000 mg/kg bw	Negative	Honarvar, 2008	Study design complies with OECD Guideline 474.
	Micronucleus Assay	Mouse bone marrow polychromatic erythrocytes	500, 1000 and 2000 mg/kg bw	Negative	Bhatia et al., 2010	Same test as Honarvar, 2008a.
2-Dodecenal [05.037]	Micronucleus Assay	Mouse bone marrow polychromatic erythrocytes	500, 1000 and 2000 mg/kg bw	Negative	Honarvar, 2007b	Study design complies with OECD Guideline 474.
	Micronucleus Assay	Mouse bone marrow polychromatic erythrocytes	500, 1000 and 2000 mg/kg bw	Negative	Bhatia et al., 2010	Same test as Honarvar, 2007b.

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ABBREVIATIONS

ABS	Chromosomal Aberrations
ALDH	Aldehyde Dehydrogenase
BrdU	Bromodeoxyuridine
bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFFA	European Flavour Association
EFSA	The European Food Safety Authority
EGF	Epidermal Growth Factor
EU	European Union
FGE	Flavouring Group Evaluation
GSH	Glutathione
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
Hex-PdG	1,N^2-propanodeoxyguanosine
HPRT	Hypoxanthine Guanine Ribosyl Transferase
ID	Identity
IOFI	International Organization of the Flavor Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
Km	Michaelis constant
LMA	Low Melting point Agarose
MF	Mutation Frequency
MN	Micronuclei
MNBN	MicroNucleated BiNucleate cells

MS	Mass spectra
MSDI	Maximised Survey-derived Daily Intake
MTD	Maximum Tolerated Dose
NMA	Normal Melting point Agarose
NMR	Nuclear Magnetic Resonance
No	Number
OECD	Organisation for Economic Co-operation and Development
PBK/D	Physiologically-Based Kinetic/Dynamic
PCE	Polychromatic Erythrocytes
PFU	Plaque-Forming Unit
PUFAs	Polyunsaturated Fatty Acids
(Q)SAR	(Quantitative) Structure Activity Relationship
RI	Replication Index
SCE	Sister Chromatid exchange
SCF	Scientific Committee on Food
TG	6-Thioguanine
TLC	Thin-Layer Chromatography
UDS	Unscheduled DNA Synthesis
Vmax	Maximal Velocity
WHO	World Health Organisation