European Food Safety Authority

The EFSA Journal (2009) 1026, 1-37

SCIENTIFIC OPINION

Flavouring Group Evaluation 61, Revision 1 (FGE.61Rev1)[1]: Consideration of aliphatic acetals evaluated by JECFA (57th meeting) structurally related to acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes and one orthoester of formic acid evaluated by EFSA in FGE.03Rev1 (2008)

Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2009-00484)

Adopted on 26 March 2009

PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on a request from the European Commission on Flavouring Group Evaluation 61 revision 1 (FGE.61rev1). *The EFSA Journal* (2009) 1026, 1-37.

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Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217 EC and its consecutive amendments.

The present consideration concerns nine aliphatic acetals evaluated by the JECFA (57th meeting) and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 58 acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes and one orthoester of formic acid evaluated in the Flavouring Group Evaluation 03, Revision 1 (FGE.03Rev1).

The Panel concluded that nine of the substances in the JECFA flavouring group of aliphatic acyclic acetals are structurally related to the 58 substances evaluated by EFSA in the FGE.03Rev1 (Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated or unsaturated aldehydes, an ester of a hemiacetal and an orthoester of formic acid).

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all nine substances considered in this FGE. However, for one substance [FL-no: 06.081] the JECFA evaluation is only based on the Maximised Survey-derived Daily Intake (MSDI) value derived from a production figure from the USA. Accordingly, the safety in use in Europe could not be assessed using the Procedure, so an EU production figure is needed in order to finalise the evaluation of this substance.

For all nine substances, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment.

In order to determine whether the conclusion for the nine JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity are available for five of the nine JECFA evaluated substances. For four substances [FL-no: 06.004, 06.005, 06.037 and 06.081] information on the stereoisomeric composition and/or composition of mixture is incomplete.

Thus, for four substances [FL-no: 06.004, 06.005, 06.037 and 06.081] the Panel has reservations (no European production volumes available, preventing evaluation using the Procedure, and/or missing data on isomerism/composition). For the remaining five substances [FL-no: 06.001, 06.008, 06.009, 06.015 and 06.028] the Panel agrees with the JECFA conclusion “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

KEYWORDS

Aliphatic acetals, JECFA, 57th meeting, acetals of branched- and straight chain aliphatic saturated primary alcohols, acetals of branched- and straight-chain saturated aldehydes, orthoester of formic acid, FGE.03Rev1.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2007, during its 55th, 57th, 59th, 61st, 63rd, 65th and 68th meetings, the JECFA evaluated about 1000 substances which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC (EC, 1999a) and its consecutive amendments.

ACKNOWLEDGEMENT

European Food Safety Authority wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Riccardo Crebelli, Karl-Heinz Engel, Henrik Frandsen, Jørn Gry, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Catherine Leclercq, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or if it has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold Criterion of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?")" (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold criterion of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the Panel evaluation could lead to a different opinion than that of the JECFA, since Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION

At its 57th meeting the JECFA evaluated a group of 10 flavouring substances consisting of aliphatic acyclic acetals. Two of the substances evaluated by the JECFA [FL-no: 06.004 and 06.005] may be metabolised to alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), they have been given special considerations in the Flavouring Group Evaluation 19 (FGE.19). The remaining seven flavouring substances have originally been considered by EFSA in the FGE.61 (EFSA, 2008z).

FGE.19 contains 360 flavouring substances from the EU Register being alpha, beta-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008b). The alpha, beta-unsaturated carbonyls were subdivided into 28 subgroups on the basis of structural similarity (EFSA, 2008b). In an attempt to

decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship ((Q)SAR) prediction of the genotoxicity of these substances was undertaken. The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni & Netzeva, 2007a; Benigni & Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. The Panel decided that 11 subgroups (1.1.2, 1.1.3, 1.1.4, 2.4, 2.6, 2.7, 3.1, 3.3, 4.1, 4.2 and 4.4) (EFSA, 2008b) should be further examined to determine whether evaluation through the Procedure is feasible. Corresponding to these 11 subgroups 11 Flavouring Group Evaluations (FGEs) were established (FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220).

History of FGE.61:

FGE	Opinion Adopted by EFSA	Link	No of Candidate Substances
FGE.61	3 July 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178700103844.htm	7
FGE.61Rev1	26 March 2009	http://www.efsa.europa.eu/EFSA/ScientificOpinionPublicationReport/efsa_locale-1178620753812_ScientificOpinions.htm	9

The present revision of FGE.61, FGE.61Rev1, includes the assessment of two additional candidate substances, citral diethyl acetal and citral dimethyl acetal [FL-no: 06.004 and 06.005] originally considered in FGE.202 (subgroup 1.1.3 in FGE.19) and for which the Panel concluded that the genotoxicity data available do not preclude their evaluation through the Procedure.

1. Presentation of the Substances in the JECFA Flavouring Group of aliphatic acetals

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 10 flavouring substances consisting of aliphatic acyclic acetals (JECFA, 2002a).

1.1.2. EFSA Considerations

Three of the JECFA evaluated acetals, citral diethyl acetal, citral dimethyl acetal and 1,1-diethoxynona-2,6-diene [FL-no: 06.004, 06.005 and 06.025], may be metabolised to alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), these three substances were given special considerations.

The remaining seven flavouring substances have originally been considered by EFSA in the FGE.61 (EFSA, 2008m).

The genotoxicity of two of the alpha,beta-unsaturated substances, citral diethyl acetal and citral dimethyl acetal [FL-no: 06.004 and 06.005], has been considered in FGE.202. The structural alert for genotoxicity is present in their metabolite citral. The Panel concluded that the data available on citral did rule out the concern for genotoxicity and thus concluded that the substances citral diethyl

acetal and citral dimethyl acetal [FL-no: 06.004 and 06.005] can be evaluated through the Procedure.

For the third substance, 1,1-diethoxynona-2,6-diene [FL-no: 06.025], considered with respect to genotoxicity in FGE.200, corresponding to subgroup 1.1.1 of FGE.19, a conclusion as to its genotoxic properties could not be reached and additional data were requested. Accordingly, this substance will not be considered in this FGE.

The present FGE.61Rev1 therefore deals with nine flavouring substances [FL-no: 06.001, 06.004, 06.005, 06.008, 06.009, 06.015, 06.028, 06.037 and 06.081].

The Panel concluded that nine of the substances in the JECFA flavouring group of aliphatic acyclic acetals are structurally related to the 58 substances evaluated by EFSA in the Flavouring Group Evaluation 03, Revision 1 (FGE.03Rev1)(Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated or unsaturated aldehydes, an ester of a hemiacetal and an orthoester of formic acid (EFSA, 2008i)). Consequently, data in FGE.03Rev1 are used to support the current consideration of the JECFA evaluation.

1.2. Isomers

1.2.1. *JECFA Status*

One flavouring substance [FL-no: 06.081] has a chiral centre and a double bond and–three substances [FL-no: 06.004, 06.005 and 06.037] have double bonds.

1.2.2. *EFSA Considerations*

Information about the stereoisomerism has not been provided for four substances [FL-no: 06.004, 06.005, 06.037 and 06.081].

1.3. Specifications

1.3.1. *JECFA Status*

The JECFA specifications are available for all nine substances (JECFA, 2001c) (see Table 1).

1.3.2. *EFSA Considerations*

Specifications are considered adequate for five substances. Information on stereoisomerism has not been provided for four substances [FL-no: 06.004, 06.005, 06.037 and 06.081] and further information on the composition of mixture of [FL-no: 06.004 and 06.005] is requested (see Section 1.2 and Table 1).

2. Intake Estimations

2.1. JECFA Status

For eight substances evaluated through the JECFA Procedure intake estimates are available for the EU (see Table 3.1). For one substance [FL-no: 06.081] production figure is only available for the USA.

2.2. EFSA Considerations

As a production figure is only available for the USA for one substance, the MSDI value for the EU cannot be calculated for this substance [FL-no: 06.081].

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[2] from the JECFA (JECFA, 2002a)

Genotoxicity data (*in vitro / in vivo*) are available for the metabolites of the nine aliphatic acyclic acetals.

In vitro

Acetaldehyde: Acetaldehyde [FL-no: 05.001] did not cause reverse mutation in the *Salmonella*/mammalian microsome assay with *S. typhmiurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without metabolic activation (Mortelmans et al., 1986). Acetaldehyde was reported to be mutagenic in mouse lymphoma cells with and without metabolic activation (Wangenheim & Bolcsfoldi, 1988). It did not cause chromosomal aberrations in normal human lymphocytes, but positive results were found in lymphocytes from a patient with Fanconi anaemia (Obe et al., 1985). Acetaldehyde increased the frequency of sister chromatid exchange in adult human lymphocytes and peripheral lymphocytes (He & Lambert, 1985; Norppa et al., 1983); however, aldehydes are rapidly oxidized to the corresponding acids and have a short plasma-life, and these important conditions that hold *in vivo* are difficult to establish *in vitro*.

Ethanol: Ethanol [FL-no: 02.078] was not mutagenic in L5178Y mouse lymphoma cells with or without metabolic activation (Wangenheim & Bolcsfoldi, 1988).

Heptanal, octanal and nonanal: [FL-no: 05.031, 05.009 and 05.025] The homologous series of aliphatic aldehydes did not induce reverse mutation in *S. typhimurium* strains (e.g. TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538) with or without metabolic activation (Florin et al., 1980; Marnett et al., 1985a; Mortelmans et al., 1986; Zeiger et al., 1992) when concentrations of up to 3333 microg/plate were used in standard (Florin et al., 1980) and preincubation (Marnett et al., 1985a; Mortelmans et al., 1986; Zeiger et al., 1992) protocols. No gene mutation was induced in a variation on the standard assay, with preincubation and metabolic activation (Mortelmans et al., 1986).

There was no evidence of unscheduled DNA synthesis when rat or human hepatocytes were incubated with nonanal at concentrations up to 100 mmol/L (Martelli et al., 1994). In standard assays, no significant increase in the frequency of chromosomal aberrations was reported when concentrations of nonanal up to 100 micromol/L (16200 microg/plate) were incubated with primary hepatocytes from Fischer 344 rats. No increase in the mitotic index or the frequency of micronuclei was seen when nonanal at 16200 microg/plate was incubated with freshly prepared rat hepatocytes (Esterbauer et al., 1990; Eckl et al., 1993). Nonanal induced a significant increase in the incidence of sister chromatid exchange in rat hepatocytes, but there was no dose–response relationship (Eckl et al., 1993).

[2] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

Decanal and octanal metabolites: Decanoic acid [FL-no: 08.011] and octanoic acid [FL-no: 08.010] (metabolites of decanal and octanal, respectively) did not induce reverse mutation in *S. typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 in the presence or absence of an exogenous metabolic activation system from the livers of Aroclor-induced male Sprague-Dawley rats and Syrian hamsters (Zeiger et al., 1988).

Citral: Citral induced mutation in *Bacillus subtilis* strains M45 and H17 at a concentration of 2.5 µL (Yoo, 1986); but no effect was seen with a concentration of 17 µg/disc (Oda et al., 1979). Citral was not mutagenic in *Escherichia coli* when tested at concentrations of 0.013–0.1 mg/plate (Yoo et al., 1986). Furthermore, it did not induce chromosomal aberrations in a Chinese hamster fibroblast cell line or reverse mutations in *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537, with and without metabolic activation (Eder et al., 1982; Lutz et al., 1982; Ishidate et al., 1984; Zeiger et al., 1987; Ishidate, 1988). It was also inactive in *S. typhimurium* (strains not specified) with metabolic activation (no further details provided) (National Toxicology Program, 1983).

In vivo

Acetaldehyde: Acetaldehyde did not cause reciprocal translocations or sex-linked recessive lethal mutation in germ cells of *Drosophila melanogaster* after oral administration; however, it induced sex-linked recessive lethal mutation when administered by injection (Woodruff et al., 1985).

Acetaldehyde administered by intraperitoneal injection to mice and hamsters induced sister chromatid exchange in bone-marrow cells (Obe et al., 1979; Korte & Obe, 1981).

Ethanol: Ethanol provided in the drinking-water of Chinese hamsters for 46 weeks at a concentration of 10 % (v/v) did not induce chromosomal aberrations or sister chromatid exchange in peripheral lymphocytes or bone-marrow cells, respectively (Korte & Obe, 1981); however, sister chromatid exchange was induced in a study in which 1.0 ml ethanol was administered by intraperitoneal injection at a concentration of 10^{-4} % (v/v) (Obe et al., 1979).

Conclusion on genotoxicity

On the basis of the results of the studies of genotoxicity, the Committee concluded that this group of aliphatic acetals is not genotoxic *in vivo*.

For a summary of *in vitro / in vivo* genotoxicity data considered by JECFA, see Table 2.1.

3.2. Genotoxicity Studies – Text from FGE.03Rev1 by EFSA (EFSA, 2008i)

In vitro / in vivo

Genotoxicity has been tested *in vitro* for three out of 58 candidate substances. These are two acetals (dimethoxymethane [FL-no: 06.074] and diethoxymethane [FL-no: 06.064]) and one orthoester of formic acid (triethoxymethane [FL-no: 06.096]). One of the acetals [FL-no: 06.074] has been tested *in vivo*. Genotoxicity data are also available for some alcohols and aldehydes resulting from hydrolysis of acetals.

Conclusion on genotoxicity

Dimethoxymethane [FL-no: 06.074] induced gene mutations in a bacterial reversion assay (Ames test) without metabolic activation but not in mammalian (CHO) cells at the HPRT locus in the presence and absence of metabolic activation. It was negative in a mouse bone marrow micronucleus assay. The studies on diethoxymethane [FL-no: 06.064] and triethoxymethane [FL-no: 06.096] were not adequately reported and the results obtained cannot be assessed. Additionally, there are some positive findings with potential hydrolysis products of acetals *in vitro* and *in vivo*, such as formaldehyde, methanol, ethanol and acetaldehyde. The genotoxicity of these compounds is well known. However, they all do occur naturally in many foods in milligram amounts (apart from alcoholic beverages) (TNO, 2000) and, based on the MSDI approach, the estimated intakes of candidate flavouring substances which might be expected to be hydrolysed to the corresponding alcohols and aldehydes are much lower.

Further, ethanol (and acetaldehyde) is endogenously synthesised. So, the daily *in vivo* formation of ethanol has been estimated to be 40-80 mg/kg body weight/day (JECFA, 1997a). Also, methanol and formaldehyde occur in milligram amounts in a number of foods (TNO, 2000) and are also endogenous metabolites. It has for instance been estimated that one cup of coffee containing 50-150 mg caffeine may give rise to the formation of about 3-7.5 mg formaldehyde in the liver (Rubach, 1987).

It is concluded that the available data on genotoxicity do not give rise to safety concern with respect to genotoxicity for the candidate flavouring substances of FGE.03Rev1 at the estimated level of intake based on MSDI.

For a summary of genotoxicity data, see in this FGE Table 2.2: Genotoxicity data (*in vitro*) EFSA / FGE.03Rev1 and Table 2.3: Genotoxicity data (*in vivo*) EFSA / FGE.03Rev1.

3.3. Genotoxicity Studies – Text from FGE.202 by EFSA (EFSA, 2008g)

Citral was not mutagenic in several valid Ames tests (Ishidate et al., 1984; Zeiger et al., 1987; Gomes-Carneiro et al., 1998; NTP, 2003e), and it did not induce chromosome aberrations in a valid *in vitro* study with chinese hamster ovary (CHO) cells (NTP, 2003e). Moreover, it was negative in a valid *in vivo* mouse bone marrow micronucleus assay (NTP, 2003e). The positive results in an *in vitro* test for sister chromatid exchanges (SCE) (NTP, 2003e) and in inappropriate test systems like the *Rec* assay in *B. subtilis* (Yoo, 1986) and the induction of the tumour suppressor protein p53 (Duerksen-Hughes et al., 1999) are considered of limited relevance for the overall evaluation. The Panel concluded that for citral genotoxicity is not of concern.

For a summary of genotoxicity data on citral, see in this FGE Table 2.4: Genotoxicity data (*in vitro*) EFSA / FGE.202 and Table 2.5 Genotoxicity data (*in vivo*) EFSA / FGE.202.

3.4. EFSA Considerations

The Panel concluded that the data available do not preclude evaluation of the nine JECFA evaluated aliphatic acyclic acetals through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to Nine Aliphatic Acyclic Acetals by JECFA (JECFA, 2002b):

According to the JECFA all nine substances belong to structural class I using the decision tree approach presented by Cramer et al. (1978).

The JECFA concluded all nine aliphatic acyclic acetals at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the threshold for structural class I (step A3).

In conclusion, the JECFA evaluated all nine substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the nine aliphatic acyclic acetals are summarised in Table 3.1: Summary of Safety Evaluation of Seven Aliphatic Acyclic Substances (JECFA, 2002a)".

4.2. Application of the Procedure to 58 Acetals of Branched- and Straight-Chain Aliphatic Saturated Primary Alcohols and Branched- and Straight-Chain Saturated Aldehydes, and One Orthoester of Formic Acid by EFSA in FGE.03Rev1 (EFSA, 2008i):

Fifty-eight candidate substances were evaluated in FGE.03Rev1. Fifty-seven substances are classified into structural class I and one substance into structural class III using the decision tree approach presented by Cramer et al. (1978).

The 58 substances were concluded at step A3, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intake is below the threshold for their structural classes I or III (step A3).

In conclusion, the Panel evaluated all 58 substances as to be of no safety concern at the estimated levels of intake as flavouring substance based on the MSDI approach.

The stepwise evaluations of the 58 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.03Rev1).

4.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all nine substances in the group of aliphatic acyclic acetals.

However, for one substance [FL-no: 06.081] no European production figure was available and consequently no European exposure estimate could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for this substance.

5. Conclusion

The present Flavouring Group Evaluation 61, Revision 1 (FGE.61Rev1) deals with nine flavouring substances [FL-no: 06.001, 06.004, 06.005, 06.008, 06.009, 06.015, 06.028, 06.037 and 06.081], evaluated by the JECFA as the flavouring group of aliphatic acyclic acetals.

The Panel concluded that the nine substances are structurally related to the 58 substances evaluated by EFSA in the Flavouring Group Evaluation 03, Revision 1 (FGE.03Rev1) (Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated or unsaturated aldehydes, an ester of a hemiacetal and an orthoester of formic acid).

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all nine substances considered in this FGE. However, for one substance [FL-no: 06.081] the JECFA evaluation is only based on the MSDI value derived from a production figure from the USA. Accordingly, the safety in use in Europe could not be assessed using the Procedure, so an EU production figure is needed in order to finalise the evaluation of this substance.

For all nine substances, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment.

In order to determine whether the conclusion for the nine substances can be applied to the materials of commerce, it is necessary to consider the available specifications. For four substances [FL-no: 06.004, 06.005, 06.037 and 06.081] information on the stereoisomeric composition and/or composition of mixture is incomplete. Adequate specifications including purity and identity are available for the five remaining substances [FL-no: 06.004, 06.005, 06.037 and 06.081].

Thus, for four substances [FL-no: 06.004, 06.005, 06.037 and 06.081] the Panel has reservations (no European production volumes available, preventing evaluation using the Procedure, and/or missing data on isomerism/composition). For the remaining five substances [FL-no: 06.001, 06.008, 06.009, 06.015 and 06.028] the Panel agrees with the JECFA conclusion “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP (JECFA, 2001C)

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Nine Aliphatic Acyclic Acetals (JECFA, 2001c)

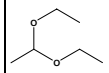
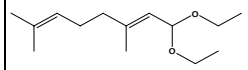
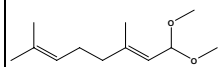
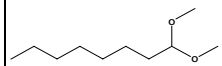
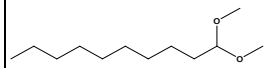
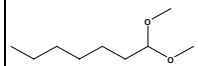
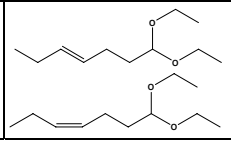
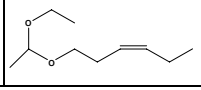
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
06.001 941	1,1-Diethoxyethane		2002 35 105-57-7	Liquid $C_6H_{14}O_2$ 118.18	Slightly soluble Miscible	102 IR 95 %	1.378-1.386 0.822-0.831	
06.004 948	Citral diethyl acetal 6)		2304 38 7492-66-2	Liquid $C_{14}H_{26}O_2$ 226.36	Insoluble Miscible	230 IR 92 %	1.445-1.455 0.864-0.879	According to JECFA: Min. assay value is "(sum of isomers + hemiacetals + citral): 98 %".
06.005 944	Citral dimethyl acetal 6)		2305 39 7549-37-3	Liquid $C_{12}H_{22}O_2$ 198.31	Insoluble Miscible	105-106 (13hPa) IR 92 %	1.450-1.463 0.881-0.893	According to JECFA: Min. assay value is "(sum of isomers + hemiacetals + citral): 98 %".
06.008 942	1,1-Dimethoxyoctane		2798 42 10022-28-3	Liquid $C_{10}H_{22}O_2$ 174.28	Insoluble Miscible	185 IR 95 %	1.410-1.420 0.841-0.851	
06.009 945	10,10-Dimethoxydecane		2363 43 7779-41-1	Liquid $C_{12}H_{26}O_2$ 202.34	Insoluble Miscible	218 IR 95 %	1.420-1.430 0.830-0.852	Register name to be changed to 1,1-dimethoxydecane.
06.015 940	1,1-Dimethoxyethane		3426 510 534-15-6	Liquid $C_4H_{10}O_2$ 90.12	Miscible Miscible	64 IR 96 %	1.365-1.367 0.850-0.860	
06.028 947	1,1-Dimethoxyheptane		2541 2015 10032-05-0	Liquid $C_9H_{20}O_2$ 160.26	Insoluble Miscible	164-165 IR 98 %	1.405-1.415 0.844-0.849	
06.037 949	1,1-Diethoxyhept-4-ene (cis and trans) 6)		3349 10011 18492-65-4	Liquid $C_{11}H_{22}O_2$ 186.29	Insoluble Miscible	93 (20 hPa) IR 97 %	1.420-1.440 0.840-0.860	CASrn in Register refers to (Z)-isomer. According to JECFA: Min. assay value is "97 % (sum of cis- and trans-isomers)".

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Nine Aliphatic Acyclic Acetals (JECFA, 2001c)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
06.081 943	1-Ethoxy-1-(3-hexenyloxy)ethane 6)		3775 10034 28069-74-1	Liquid $C_{10}H_{20}O_2$ 172.27	Insoluble Miscible	85 (9 hPa) IR 97 %	1.430-1.435 0.846-0.856	CASrn in Register refers to (Z)-isomer.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95% ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro / in vivo*) for the Metabolites of Nine Aliphatic Acyclic Acetals (JECFA, 2002a)

Table 2.1: Summary of Genotoxicity Data for the Metabolites of Nine Aliphatic Acyclic Acetals (JECFA, 2002a)

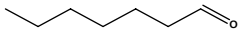
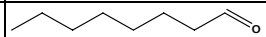
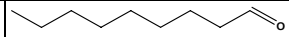
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
05.001	Acetaldehyde		Reverse mutation (preincubation)	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	10 mg/plate	Negative[a]	(Mortelmans et al., 1986)
			Chromosomal aberration	Human lymphocytes	0.002% (v/v)	Negative[b]	(Obe et al., 1979)
			Sister chromatid exchange	Human lymphocytes	2.4 mmol/L	Positive[c]	(He & Lambert, 1985)
			Sister chromatid exchange	Human lymphocytes	2 mmol/L	Positive[d]	(Norppa et al., 1983)
			Mutation cells	L5178Y mouse lymphoma	8.0×10^{-3} mol/L	Positive[e]	(Wangenheim & Bolcsfoldi, 1988)
02.078	Ethanol		Mutation cells	L5178Y mouse lymphoma	7.4×10^{-1} mol/L	Negative[a]	(Wangenheim & Bolcsfoldi, 1988)
05.031	Heptanal		Reverse mutation (spot test)	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative[a]	(Florin et al., 1980)
			Reverse mutation (preincubation)	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	1–3300 µg/plate	Negative[a]	(Zeiger et al., 1992)
05.009	Octanal		Reverse mutation (spot test)	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative[a]	(Florin et al., 1980)
05.025	Nonanal		Reverse mutation (spot test)	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative[a]	(Florin et al., 1980)
			Sister chromatid exchange	Female Fischer 344 rat hepatocytes	0.1–100 µmol/L	Positive[f]	(Eckl et al., 1993)
			Unscheduled DNA synthesis	Adult human and rat hepatocytes	3–100 mmol/L	Negative[g]	(Martelli et al., 1994)
			Gene mutation (preincubation)	*S. typhimurium* TA98, TA100, TA1535	1–670 µg/plate	Negative[h]	(Mortelmans et al., 1986)
			Reverse mutation (liquid preincubation)	*S. typhimurium* TA102, TA104	1 mg/plate	Negative	(Marnett et al., 1985a)

Table 2.1: Summary of Genotoxicity Data for the Metabolites of Nine Aliphatic Acyclic Acetals (JECFA, 2002a)

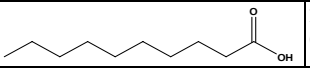
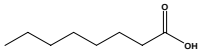
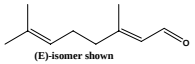
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Micronucleus formation	Female Fischer 344 rat hepatocytes	0.1–100 µmol/L	Negative	(Esterbauer et al., 1990)
			Micronucleus formation	Female Fischer 344 rat hepatocytes	0.1–100 µmol/L	Negative	(Eckl et al., 1993)
			Chromosomal aberration	Female Fischer 344 rat hepatocytes	0.1–100 µM	Negative	(Esterbauer et al., 1990)
			Chromosomal aberration	Female Fischer 344 rat hepatocytes	0.1–100 µM	Negative	(Eckl et al., 1993)
08.011	Decanoic acid		Reverse mutation (preincubation)	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0.05 ml/plate	Negative[a]	(Zeiger et al., 1988)
08.010	Octanoic acid		Reverse mutation (preincubation)	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0.05 ml/plate	Negative[a]	(Zeiger et al., 1988)
05.020	Citral	 (E)-isomer shown	Reverse mutation (preincubation)	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98	0.1 mg/plate	Negative[a]	Ishidate et al. (1984)
			Reverse mutation (preincubation)	*S. typhimurium* TA100	NR	Negative[a]	Eder et al. (1982)
			Reverse mutation (preincubation)	*S. typhimurium* TA100	NR	Negative[a]	Lutz et al. (1982)
			Reverse mutation (preincubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	160 µg/plate	Negative[a]	Zeiger et al. (1987)
			Reverse mutation (preincubation)	*S. typhimurium* (strains not specified)	NR	Negative[h]	National Toxicology Program (1983)
			Mutation	*Escherichia coli WP2 uvrA (trp⁻)*	0.1 mg/plate	Negative[i]	Yoo (1986)
			Gene mutation	*Bacillus subtilis* M45 and H17 *rec*	17 µg/disc	Negative[i]	Oda et al. (1979)
			Gene mutation	*B. subtilis* M45 and H17 *rec*	2.5 µl/disc	Positive[i]	Yoo (1986)
			Chromosomal aberration	Chinese hamster fibroblasts	0.03 mg/ml	Negative[a]	Ishidate et al. (1984)

Table 2.1: Summary of Genotoxicity Data for the Metabolites of Nine Aliphatic Acyclic Acetals (JECFA, 2002a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Chromosomal aberration	Chinese hamster fibroblasts	30 µg/ml	Negative[e]	Ishidate (1988)
In vivo							
05.001	Acetaldehyde		Reciprocal translocation	*Drosophila melanogaster*	0.05 ml/vial	Negative[j]	(Woodruff et al., 1985)
			Sex-linked recessive lethal mutation	*D. melanogaster*	0.05 ml/vial	Negative[j]	(Woodruff et al., 1985)
			Sex-linked recessive lethal mutation	*D. melanogaster*	0.3 µl	Positive[k]	(Woodruff et al., 1985)
			Sister chromatid exchange	Chinese hamster bone-marrow cells	0.5 mg/kg bw	Positive[l]	(Korte & Obe, 1981)
			Sister chromatid exchange	Mouse bone marrow cells	20% (v/v)	Positive[l]	(Obe et al., 1979)
02.078	Ethanol		Chromosomal aberration	Chinese hamster peripheral lymphocytes	10% (v/v)	Negative[m]	(Korte & Obe, 1981)
			Sister chromatid exchange	Chinese hamster bone marrow cells	10% (v/v)	Negative[m]	(Korte & Obe, 1981)
			Sister chromatid exchange	Mouse bone marrow cells	1.0 ml of 10–4% (v/v)	Positive[l]	(Obe et al., 1979)

[a] *Assay performed with and without S9.*

[b] *Positive results with lymphocytes from patient with Fanconi anaemia.*

[c] *Cells exposed for various times in various phases of cell cycle.*

[d] *Abstract.*

[e] *Assay performed without S9.*

[f] *No dose–response relationship.*

[g] *20-h exposure.*

[h] *Assay performed with S9.*

[i] *Japanese article, English summary and tables.*

[j] *Administered orally.*

[k] *Administered by injection.*

[l] *Administered by intraperitoneal injection.*

[m] *Given in drinking-water for 46 weeks.*

Table 2.2: Genotoxicity (*in vitro*) EFSA / FGE.03Rev1

Substances in brackets are JECFA evaluated substances

Table 2.2: Summary of genotoxicity data (*in vitro)* EFSA / FGE.03Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Dimethoxymethane [06.074]	Ames test	*S. typhimurium*. TA98, TA 100 *S. typhimurium* TA 1535, TA 1537, TA 1538	667-10000 microgram/plate 667-10000 microgram/plate	Neg.* / Pos.** Neg.*/** (See footnote 1)	(Hoechst-Celanese Corp., 1989b)	In compliance with GLP and OECD guideline 471 (1983).
	HGPRT assay	CHO cells	0.5 to 5 mg/l	Neg.*/** (See footnote 2)	(Hoechst-Celanese Corp., 1990a)	In compliance with GLP and OECD guideline 476 (1984).
Diethoxymethane [06.064]	Ames test	*S. typhimurium*. TA98, TA 100, TA 1535, TA 1537, TA 1538	100-10000 microgram/plate	Neg.*/** (See footnote 3)	(Cameron, 1995)	Quality of studies cannot be evaluated.
	Mouse lymphoma TK assay	L5178Y (TK+/TK-)	3,000 – 5,000 microgram/ml 250 – 1500 microgram/ml	Neg.** Pos.* (See footnote 3)	(Cameron, 1995)	Quality of studies cannot be evaluated.
Triethoxymethane [06.096]	Ames test	*S. typhimurium* TA97, TA98, TA 100	8 – 5,000 microgram/plate	Neg.*/** (See footnote 3)	(Huels, 1992)	Quality of studies cannot be evaluated.

**With metabolic activation.*

***Without metabolic activation.*

1) Dimethoxymethane [06.074] (purity not reported) was tested in a bacterial reversion assay (Ames test) with Salmonella typhimurium *strains TA98, TA100, TA1535, TA1537 and TA1538 with and without exogenous metabolic activation (liver S9 mix from rats pretreated with Aroclor 1254), following the preincubation method. A dose range-finding experiment was performed with strain TA100 at doses from 10 to 10000 microgram/plate (one plate per dose). The main experiment was conducted at five doses from 667 to 10000 microgram/plate. All doses were tested in triplicate. Water was used as solvent.*

Result: A weak positive response was observed with strain TA100 in the absence of microsomal enzymes (2.4-fold maximum increase in revertant colonies in the dose range-finding experiment and 2.1-fold maximum increase in the main study at 10000 microgram/plate, respectively). A positive response was also observed with strain TA98 in the absence of microsomal enzymes (3.9-fold maximum increase at 10000 microgram/plate). These effects were dose-related. No positive responses were observed with any of the other strains and activation conditions. No bacteriotoxicity was observed up to 10000 microgram/plate in the presence and absence of microsomal enzymes. Precipitations were not observed.

2) Dimethoxymethane [06.074] (purity not reported) was tested in a gene mutation assay at the HPRT locus in the CHO-K1-BH_4 Chinese Hamster Ovary (CHO) cell line with and without exogenous metabolic activation (liver S9 mix from rats pretreated with Aroclor 1254). A dose range-finding experiment was performed with 10 concentrations from 0.0098 to 5.0 mg/ml. One main experiment was performed with six dose levels from 0.5 to 5.0 mg/ml. Duplicate cell cultures were used for each experimental point. Water was used as solvent.

Result: The test substance produced slight toxicity at concentrations above 1.0 mg/ml in the assays with and without metabolic activation. One treated culture each with and without metabolic activation had a mutant frequency that was statistically elevated over the mutant frequencies of the concurrent vehicle control cultures. Adjacent dose levels with similar levels of toxicity showed no indication of a mutagenic response. The significant mutant frequencies were within the normal range for background mutant frequency variation which was 0 to 15 x 10^{-6}. The test substance was considered negative for inducing forward mutations at the HPRT locus in CHO cells.

3) There are data on genotoxicity for diethoxymethane [06.064] and triethoxymethane [06.096]. While diethoxymethane [06.064] is reported to be negative in a bacterial reversion assay (Ames test) it is reported to be positive in a gene mutation assay at the TK locus in mammalian cells in the presence of metabolic activation (Cameron, 1995). Triethoxymethane [06.096] is reported to be negative in a bacterial reversion assay (IUCLID data base of the European Chemicals Bureau, referring on Huels Report No. AM-92/20, 1992 (unpublished) (Report is not available)). However, from these studies, details are not available with respect to methods and results, respectively. Thus, the quality of these studies cannot be evaluated.

Table 2.3: Genotoxicity (*in vivo*) EFSA / FGE.03Rev1

Substances in brackets are JECFA evaluated substances

Table 2.3: Summary of genotoxicity data (*in vivo)* EFSA / FGE.03Rev1

Chemical Name [FL-no]	Test system	Test Object	Route	Dose	Result	Reference	Comments
Dimethoxymethane [06.074]	Micronucleus assay	Mouse	I.p.	400 – 4,000 mg/kg bw	Negative (See footnote 1)	(Hoechst-Celanese Corp., 1990b)	In compliance with GLP and OECD guideline 474 (1983).

1) Dimethoxymethane [06.074] (purity not reported) was tested in the micronucleus test in bone marrow cells of ICR mice. Based on the results of a previously conducted dose range-finding study, groups of five males and five females were exposed to the test substance at doses of 400, 1333, and 4000 mg/kg body weight by intraperitoneal injection (0.9 % sodium chloride was used as vehicle). The animals were sacrificed 24, 48 and 72 hours after dosing. Micronuclei were scored in 1000 PCEs per animal. The PCE/NCE ratio was determined by scoring the number of NCEs while scoring 1000 PCEs.

Result: Within one minute of dosing mice at the 4000 mg/kg dose became prostrate with dyspnea and mice at 1333 mg/kg showed uncoordinated movement. Most mice recovered in one hour. The PCE/NCE ratio was reduced in single groups (e.g. 0.59 at 4000 mg/kg after 24 hours in males) however, the PCE/NCE ratio was not clearly dose-related. The test substance did not induce a significant increase in micronucleated bone marrow PCEs.

Table 2.4: Genotoxicity (*in vivo*) EFSA / FGE.202

Table 2.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments
Citral [05.020]	Reverse mutation	*S. typhimurium* TA98, TA100, TA97a, TA102	5–700 µg/plate	Negative[a]	(Gomes-Carneiro et al., 1998)	Published non-GLP study containing sufficient details. Result is considered as valid.
	Reverse mutation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	Up to 100 µg/plate	Negative[b]	(Ishidate et al., 1984)	According to current guidelines. The study is considered valid.
	Reverse mutation	*S. typhimurium* TA100	NR	Negative[a]	(Lutz et al., 1982)	One strain only. Concentrations tested not specified. No re-run of the test; no other data on experimental results or design apart from a description of the test method. Validity cannot be evaluated.
	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–160 µg/plate	Negative[a]	(Zeiger et al., 1987) (NTP, 2003e)	Valid. Standard NTP study carried out according to US EPA guidelines; result is considered valid.
	Mutation	*E. coli* WP2uvrA (trp -)	13–100 µg/plate	Negative	(Yoo, 1986)	(Study in Japanese). Validity cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	0.289–40.2 µg/ml	Positive[a]	(NTP, 2003e)	Standard NTP study carried out according to US EPA guidelines; result is considered valid.
	Chromosomal aberration	Chinese hamster ovary cells	12.5–60.6 µg/ml	Negative[a]	(NTP, 2003e)	Standard NTP study carried out according to US.EPA guidelines; Result is considered valid.
	Chromosomal aberration	Chinese hamster fibroblast cells	Up to 30 µg/ml	Negative[c]	(Ishidate et al., 1984)	Limited validity (performed only in the presence of metabolic activation).
	Rec assay	*B. subtilis* M45 and H17	17 µg/disk	Negative	(Oda et al., 1979)	The test system used is considered inappropriate; insufficient validity.
	Rec assay	*B. subtilis* M45 and H17	0.16, 0.32, 0.63 µl/disk (142, 284, 560 µg/disk)[d] 1.25, 2.5 µl/disk (1110, 2220 µg/disk)[d]	Negative Positive	(Kuroda et al., 1984a)	Article in Japanese; with limited information in tables and abstract. Assay of limited relevance. Validity cannot be evaluated.
	Rec assay	*B. subtilis* M45 and H17	<2.5 µl/disk (<2220 µg/disk)	Positive	(Yoo, 1986)	(Study in Japanese). Study with limited relevance Validity cannot be evaluated.
	Induction of tumour suppressor protein p53 (DNA damage)	Mouse fibroblast cells (NTCT 929)	10–30 µg/ml	Positive	(Duerksen-Hughes et al., 1999)	The Induction of tumor suppressor protein p53 may be considered as indicator for genotoxicity. Result is considered valid, however, it has only limited relevance.

NR not reported.

[a] *With and without metabolic activation*

[b] *With metabolic activation.*

[c] *Without metabolic activation.*

[d] *Calculated using a density of 0.888 (Merck, 1997).*

Table 2.5: Genotoxicity (*in vivo*) EFSA / FGE.202

Table 2.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
Citral [05.020]	Micronucleus formation	Mouse bone marrow erythrocytes	Three intraperitoneal injections given at 24-h intervals; male mice only	250, 500, or 750 mg/kg bw	Negative	(NTP, 2003e)	NTP study carried out according to US EPA guideline. Result is considered as valid.
	Micronucleus formation	Mouse peripheral blood erythrocytes	Microencapsulated citral was administered in the diet for 14 weeks	745, 1840, 3915, or 8110 mg/kg bw per day (males) 790, 1820, 3870, or 7550 mg/kg bw per day (females)	Negative Negative	(NTP, 2003e)	NTP study carried out according to a non-standard guideline; result is considered of limited validity.

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of Nine Aliphatic Acyclic Acetals (JECFA, 2002a)

Table 3.1: Summary of Safety Evaluation of nine JECFA Evaluated Aliphatic Acyclic Acetals (JECFA, 2002a)

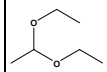
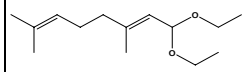
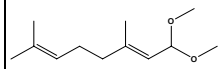
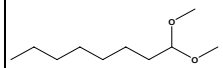
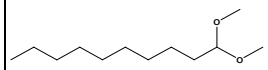
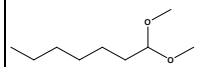
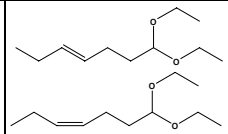
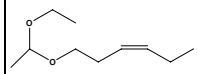
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
06.001 941	1,1-Diethoxyethane		200 640	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
06.004 948	Citral diethyl acetal		3.4 0	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	Stereoisomeric composition and composition of mixture to be specified.
06.005 944	Citral dimethyl acetal		2.6 5	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	Stereoisomeric composition and composition of mixture to be specified.
06.008 942	1,1-Dimethoxyoctane		0.97 0	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
06.009 945	10,10-Dimethoxydecane		0.024 0	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	Register name to be changed to: 1,1-dimethoxydecane. No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
06.015 940	1,1-Dimethoxyethane		61 11	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of nine JECFA Evaluated Aliphatic Acyclic Acetals (JECFA, 2002a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
06.028 947	1,1-Dimethoxyheptane		0.037 0.26	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
06.037 949	1,1-Diethoxyhept-4-ene (cis and trans)		0.037 0	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.	CASrn refers to (Z)-isomer. According to the JECFA: Min. assay value is "97 % (sum of cis- and trans-isomers)". Stereoisomeric composition to be specified.
06.081 943	1-Ethoxy-1-(3-hexenyloxy)ethane		ND 0	Class I A3: Intake below threshold	4)	MSDI based on USA production figure.	CASrn refers to (Z)-isomer, Stereoisomeric composition to be specified. MSDI based on USA production figure.

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

ND: not determined.

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.03Rev1)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

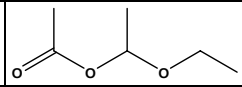
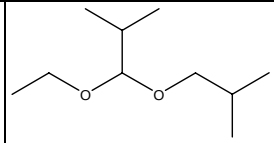
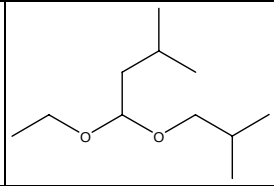
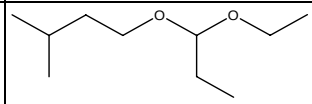
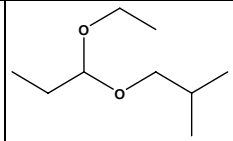
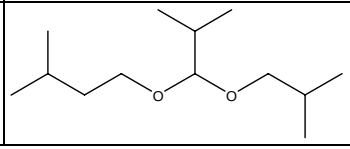
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
03.023	1-Ethoxyethyl acetate		7.1	Class I A3: Intake below threshold	4)	7)	
06.041	1-Isobutoxy-1-ethoxy-2-methylpropane		0.012	Class I A3: Intake below threshold	4)	7)	
06.042	1-Isobutoxy-1-ethoxy-3-methylbutane		0.012	Class I A3: Intake below threshold	4)	7)	
06.043	1-Isoamyloxy-1-ethoxypropane		0.012	Class I A3: Intake below threshold	4)	7)	
06.044	1-Isobutoxy-1-ethoxypropane		0.012	Class I A3: Intake below threshold	4)	7)	
06.045	1-Isobutoxy-1-isopentyloxy-2-methylpropane		0.012	Class I A3: Intake below threshold	4)	7)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

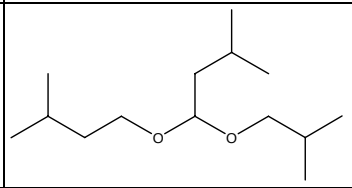
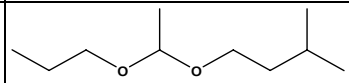
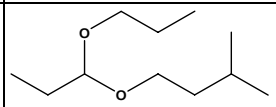
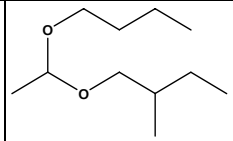
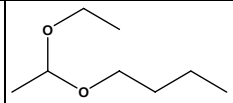
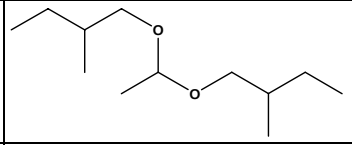
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.046	1-Isobutoxy-1-isopentyloxy-3-methylbutane		0.012	Class I A3: Intake below threshold	4)	7)	
06.047	1-Isopentyloxy-1-propoxyethane		0.037	Class I A3: Intake below threshold	4)	7)	
06.048	1-Isopentyloxy-1-propoxypropane		0.012	Class I A3: Intake below threshold	4)	7)	
06.049	1-Butoxy-1-(2-methylbutoxy)ethane		0.0061	Class I A3: Intake below threshold	4)	7)	
06.050	1-Butoxy-1-ethoxyethane		0.012	Class I A3: Intake below threshold	4)	7)	
06.051	1,1-Di-(2-methylbutoxy)ethane		0.012	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

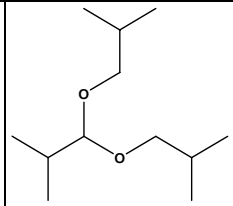
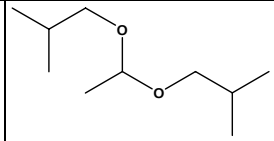
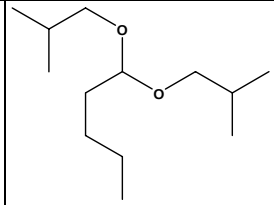
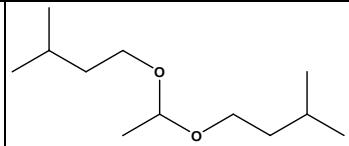
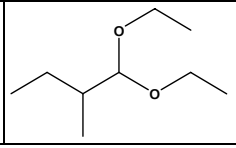
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.052	1,1-Di-isobutoxy-2-methylpropane		0.39	Class I A3: Intake below threshold	4)	6)	
06.053	1,1-Di-isobutoxyethane		0.13	Class I A3: Intake below threshold	4)	6)	
06.054	1,1-Di-isobutoxypentane		0.12	Class I A3: Intake below threshold	4)	6)	
06.055 1729	1,1-Di-isopentyloxyethane		14	Class I A3: Intake below threshold	4)	6)	
06.057	1,1-Diethoxy-2-methylbutane		0.73	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

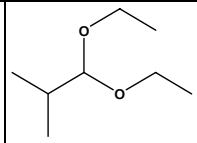
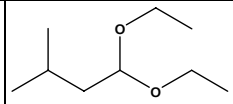
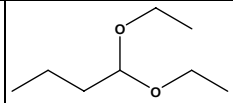
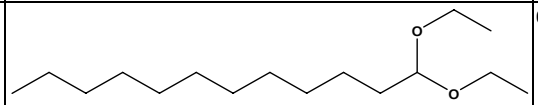
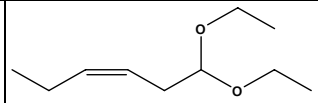
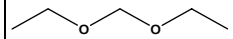
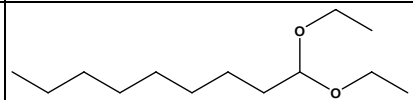
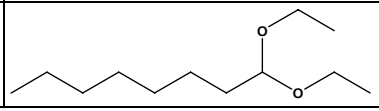
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.058	1,1-Diethoxy-2-methylpropane		0.67	Class I A3: Intake below threshold	4)	6)	
06.059 1730	1,1-Diethoxy-3-methylbutane		0.51	Class I A3: Intake below threshold	4)	6)	
06.061	1,1-Diethoxybutane		0.69	Class I A3: Intake below threshold	4)	6)	
06.062	1,1-Diethoxydodecane		0.37	Class I A3: Intake below threshold	4)	6)	
06.063	1,1-Diethoxyhex-3-ene	 **Z-form shown**	0.097	Class I A3: Intake below threshold	4)	7)	
06.064	Diethoxymethane		0.097	Class I A3: Intake below threshold	4)	6)	
06.065	1,1-Diethoxynonane		0.52	Class I A3: Intake below threshold	4)	6)	
06.066	1,1-Diethoxyoctane		0.0012	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

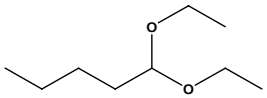
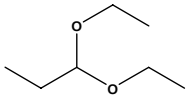
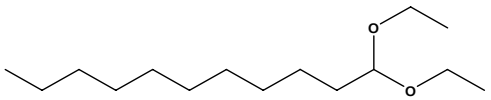
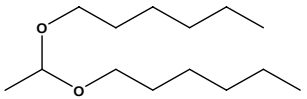
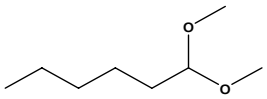
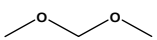
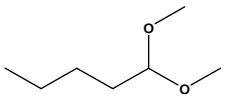
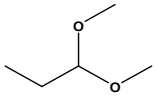
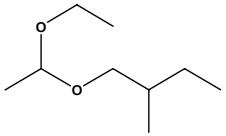
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.067	1,1-Diethoxypentane		0.12	Class I A3: Intake below threshold	4)	6)	
06.069	1,1-Diethoxypropane		0.77	Class I A3: Intake below threshold	4)	6)	
06.070	1,1-Diethoxyundecane		0.0012	Class I A3: Intake below threshold	4)	6)	
06.071	1,1-Dihexyloxyethane		0.67	Class I A3: Intake below threshold	4)	6)	
06.073	1,1-Dimethoxyhexane		0.56	Class I A3: Intake below threshold	4)	6)	
06.074	Dimethoxymethane		0.012	Class I A3: Intake below threshold	4)	6)	
06.075	1,1-Dimethoxypentane		0.73	Class I A3: Intake below threshold	4)	6)	
06.076	1,1-Dimethoxypropane		0.12	Class I A3: Intake below threshold	4)	6)	
06.079	1-Ethoxy-1-(2-methylbutoxy)ethane		0.073	Class I A3: Intake below threshold	4)	7)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

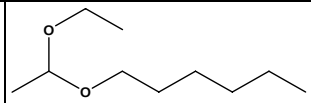
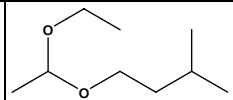
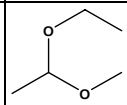
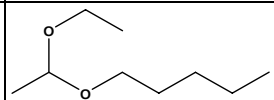
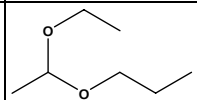
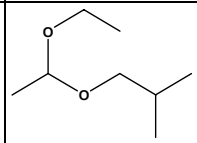
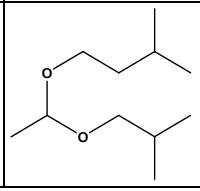
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.082	1-Ethoxy-1-hexyloxyethane		0.37	Class I A3: Intake below threshold	4)	7)	
06.083	1-Ethoxy-1-isopentyloxyethane		1.2	Class I A3: Intake below threshold	4)	7)	
06.084	1-Ethoxy-1-methoxyethane		0.12	Class I A3: Intake below threshold	4)	7)	
06.085	1-Ethoxy-1-pentyloxyethane		0.012	Class I A3: Intake below threshold	4)	7)	
06.086	1-Ethoxy-1-propoxyethane		0.012	Class I A3: Intake below threshold	4)	7)	
06.091	1-Isobutoxy-1-ethoxyethane		0.097	Class I A3: Intake below threshold	4)	7)	
06.092	1-Isobutoxy-1-isopentyloxyethane		0.37	Class I A3: Intake below threshold	4)	7)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

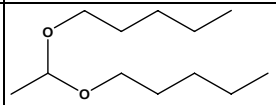
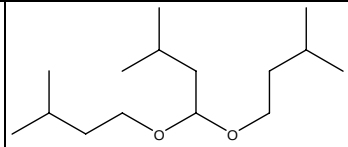
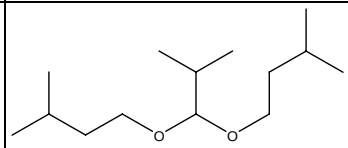
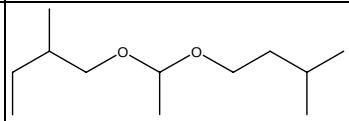
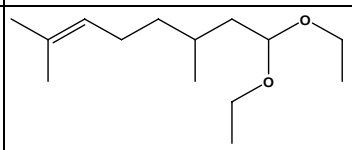
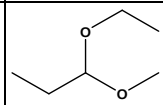
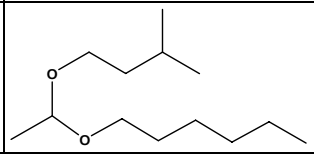
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.100	1,1-Dipentyloxyethane		0.85	Class I A3: Intake below threshold	4)	6)	
06.105	3-Methyl-1,1-di-isopentyloxybutane		0.012	Class I A3: Intake below threshold	4)	7)	
06.106	2-Methyl-1,1-di-isopentyloxypropane		0.26	Class I A3: Intake below threshold	4)	7)	
06.107	1-(2-Methylbutoxy)-1-isopentyloxyethane		0.024	Class I A3: Intake below threshold	4)	7)	
06.109	1,1-Diethoxy-3,7-dimethyloct-6-ene		0.24	Class I A3: Intake below threshold	4)	7)	
06.111	1-Ethoxy-1-methoxypropane		0.012	Class I A3: Intake below threshold	4)	7)	
06.114	1-Hexyloxy-1-isopentyloxyethane		0.0	Class I A3: Intake below threshold	4)	7)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

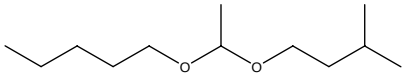
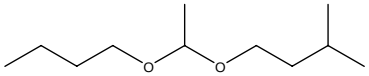
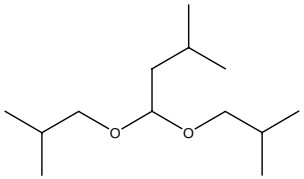
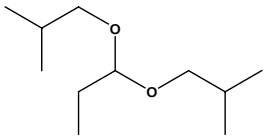
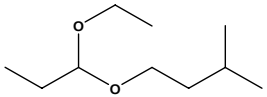
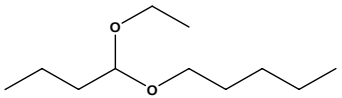
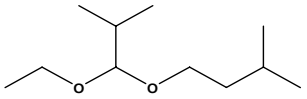
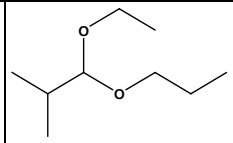
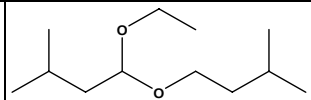
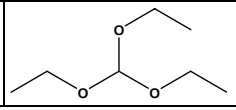
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.115	1-Isopentyloxy-1-pentyloxyethane		0.24	Class I A3: Intake below threshold	4)	7)	
06.123	1-Butoxy-1-isopentyloxyethane		0.0061	Class I A3: Intake below threshold	4)	7)	
06.124	1,1-Di-isobutoxy-3-methylbutane		0.037	Class I A3: Intake below threshold	4)	7)	
06.125	1,1-Di-isobutoxypropane		0.37	Class I A3: Intake below threshold	4)	6)	
06.127	1-Ethoxy-1-isopentyloxypropane		0.012	Class I A3: Intake below threshold	4)	7)	
06.128	1-Ethoxy-1-pentyloxybutane		0.012	Class I A3: Intake below threshold	4)	7)	
06.129	1-Ethoxy-2-methyl-1-isopentyloxypropane		0.012	Class I A3: Intake below threshold	4)	7)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.130	1-Ethoxy-2-methyl-1-propoxypropane		0.012	Class I A3: Intake below threshold	4)	7)	
06.131	1-Ethoxy-1-(3-methylbutoxy)-3-methylbutane		0.012	Class I A3: Intake below threshold	4)	7)	
06.096	Triethoxymethane		0.013	Class III A3: Intake below threshold	4)	6)	

1) MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach)

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

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ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practise
ID	Identity
Ip	Intraperitoneal
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation