EFSA ; Scientific Opinion on Flavouring Group Evaluation 59, Revision 1 (FGE.59Rev1): Consideration of aliphatic and aromatic ethers evaluated by JECFA (61st meeting and 63rd meeting) structurally related to aliphatic, alicyclic and aromatic ethers including anisole derivatives evaluated by EFSA in FGE.23 Rev2 (2010)

EFSA Publication; Larsen, John Christian; Nørby, Karin Kristiane; Beltoft, Vibe Meister; Lund, Pia; Binderup, Mona-Lise; Frandsen, Henrik Lauritz

Link to article, DOI:
10.2903/j.efsa.2011.2158

Publication date:
2011

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

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 59, Revision 1 (FGE.59Rev1):

Consideration of aliphatic and aromatic ethers evaluated by JECFA (61st meeting and 63rd meeting) structurally related to aliphatic, alicyclic and aromatic ethers including anisole derivatives evaluated by EFSA in FGE.23 Rev2 (2010)[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 consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 30 flavouring substances consisting of aliphatic and aromatic ethers evaluated by the JECFA. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for the 30 substances considered in this FGE and agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for two substances, are information on the composition of stereoisomeric mixture lacking.

1 On request from the Commission, Question No EFSA-Q-2011-00051, adopted on 24 March 2011.

2 Panel members: Arturo Anadon, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle. cef-unit@efsa.europa.eu

3 Acknowledgement: The Panel 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, Karl-Heinz Engel, Henrik Frandsen, Jørn Gry, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific opinion.

Suggested citation: EFSA ; Scientific Opinion on Flavouring Group Evaluation 59, Revision 1 (FGE.59Rev1): Consideration of aliphatic and aromatic ethers evaluated by JECFA (61st meeting and 63rd meeting) structurally related to aliphatic, alicyclic and aromatic ethers including anisole derivatives evaluated by EFSA in FGE.23 Rev2 (2010). EFSA Journal 2011; 9(5):2158. [32 pp.]. doi:10.2903/j.efsa.2011.2158. Available online: www.efsa.europa.eu/efsajournal.htm

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

The present consideration concerns a group of 30 flavouring substances consisting of aliphatic and aromatic ethers by the JECFA (61st and 63rd meeting) and will be considered in relation to the EFSA evaluation of 19 aliphatic, alicyclic and aromatic ethers including anisole derivatives of evaluated in the Flavouring Group Evaluation 23, Revision 2 (FGE.23Rev2).The Panel concluded that all the 30 substances in the JECFA flavouring group of aliphatic and aromatic ethers are structurally related to the group of 19 aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30 evaluated by EFSA in the Flavouring Group Evaluation 23, Revision 2 (FGE.23Rev2).

The Panel agrees with the application of the Procedure as performed by the JECFA for the 30 substances considered in this FGE.

For all 30 substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment to finalise the evaluation.

In order to determine whether the conclusion for the 30 JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for 28 of the 30 JECFA evaluated substances. For two substances [FL-no: 13.037 and 13.072], have the composition of the mixture of diastereoisomers not been specified sufficiently. Thus, for the two substances [FL-no: 13.037 and 13.072], the Panel has reservations (information on stereoisomeric composition is missing).

For the remaining 28 of the 30 JECFA evaluated aliphatic and aromatic ethers [FL-no: 03.001, 03.003, 03.004, 03.005, 03.006, 03.007, 03.010, 03.019, 04.014, 04.015, 04.016, 04.032, 04.033, 04.034, 04.035, 04.038, 04.039, 04.040, 04.043, 04.054, 04.062, 04.063, 04.074, 13.088, 13.094, 13.098, 13.165 and 16.088] the Panel agrees with the JECFA conclusion “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

KEY WORDS

Aliphatic and aromatic ethers, JECFA, 61st meeting, aliphatic, alicyclic and aromatic ethers, anisole derivatives, chemical groups 15, 16, 26, FGE.59, FGE 23Rev2.

TABLE OF CONTENTS

BACKGROUND

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

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

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 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th 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.

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, 1999a), 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 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 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 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 of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999a), 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 evaluation by the Panel could lead to a different opinion than that of JECFA, since the 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 OF THE SUBSTANCES IN THE PRESENT FGE

At its 61st meeting the JECFA evaluated a group of 29 flavouring substances consisting of aliphatic and aromatic ethers. These substances have been considered by EFSA in FGE.59 (EFSA, 2008ac).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
59	3 July 2007	http://www.efsa.europa.eu/en/scdocs/scdoc/639.htm	29
59Rev1	22 March 2011		30

At its 63rd meeting the JECFA evaluated a group of 32 monocyclic and bicyclic secondary alcohols, ketones and related esters including one aliphatic ether, l-menthyl methyl ether [FL-no: 16.088]. The Panel concluded that this ether should be considered together with the ethers from FGE.59 in FGE.59 Revision 1 (FGE.59Rev1). This consideration will therefore deal with 30 JECFA evaluated aliphatic and aromatic ethers.

In FGE.59, information on stereoisomerism or composition of mixture of isomers was requested for the following substances: [FL no: 03.005, 13.037, 13.072, 13.088, 13.094, 13.098, 13.165, 03.007 and 03.010]. Industry has submitted additional information on the specifications for these substances, which has been taken into consideration in this revision of FGE.59. Sufficiently information was not submitted for [FL-no: 13.037 and 13.072].

In FGE.59 seven substances [FL-no: 03.010, 04.033, 04.040, 04.062, 04.063, 04.074 and 13.165] could not be evaluated using the Procedure, because no EU production figures were available. In the course of 2010, Industry provided EU production figures for these seven substances together with similar data on approximately 100 other substances from 27 different FGEs. In order to avoid unnecessary delay, these substances were evaluated in a special FGE, FGE.96, in which EU production volumes / anticipated production volumes submitted on request by DC SANCO have been included in the evaluation (EFSA, 2010aj). The EU production volumes of these seven substances and the outcome of the evaluations from FGE.96 have also been included in the current revision of FGE.59.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 29 flavouring substances consisting of aliphatic and aromatic ethers (JECFA, 2004b) and one aliphatic ether, *l*-menthyl methyl ether [FL-no: 16.088], in the group of monocyclic and bicyclic alcohols, ketones and related esters (JECFA, 2006d).

1.1.2. EFSA Considerations

The Panel concluded that these 30 substances are structurally related to the group of 19 aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30 evaluated by EFSA in Flavouring Group Evaluation 23, Revision 2 (FGE.23Rev2).

1.2. Isomers

1.2.1. Status

The following four JECFA-evaluated substances have one chiral centre [FL-no: 03.005, 13.088, 13.094 and 13.165], two substances have two chiral centres [FL-no: 13.037 and 13.098], one substance has three chiral centres [FL-no: 16.088] and one substance has four chiral centres [FL-no: 13.072].

1.2.2. EFSA Considerations

The steroisomeric composition has been specified for [FL-no: 03.005, 13.088, 13.094, 13.098, 13.165 and 16.088]. For [FL-no: 13.037 and 13.072], Industry has informed that it occurs as a mixture of diastereoisomers (EFFA, 2010a), however, the composition of the mixture has to be specified (see Table 1).

1.3. Specifications

1.3.1. JECFA Status

JECFA specifications are available for all 30 substances (JECFA, 2003b; JECFA, 2005b), see Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate for 28 substances. For two substances [FL-no: 13.037 and 13.072] information on the composition of stereoisomeric mixture is lacking (see Section 1.2).

2. Intake Estimations

2.1. JECFA Status

For all 30 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1.

2.2. EFSA Considerations

No comments

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[4] from the JECFA (JECFA, 2004b)

No information on [FL-no: 16.088] or other ethers is available from the JECFA evaluation of 32 monocyclic and bicyclic secondary alcohols, ketones and related esters including one aliphatic ether (JECFA, 2006a).

In vitro

Negative results were reported in the standard Ames assay when various strains of *Salmonella typhimurium* (TA97, TA98, TA100, TA102, TA1532, TA1535, TA1537, TA1538, TA1978 and TA2636) were incubated with eucalyptol [FL-no: 03.001 (1,8-cineole)], anisole [FL-no: 04.032], *p*-methylanisole [FL-no: 04.015 (1-Methoxy-4-methylbenzene)], *p*-propylanisole [FL-no: 04.039 (1-methoxy-4-propylbenzene)], 1,2-dimethoxybenzene [FL-no: 04.062], *m*-dimethoxybenzene [FL-no: 04.016)1,3-dimethoxybenzene)], *p*-dimethoxy-benzene [(FL-no: 04.034 (1,4-dimethoxybenzene)], diphenyl ether [FL-no: 04.035], dibenzyl ether [FL-no: 03.004], *beta*-naphthyl methyl ether [FL-no: 04.074 (2-methoxynaphtalene)], *beta*-naphthyl ethyl ether [FL-no: 04.033], or *beta*-naphthyl isobutyl ether [FL-no: 04.054 (isobutyl beta- naphthyl ether)] at concentrations of up to 50 000 µg/plate, with and without metabolic activation (Clark et al., 1979; Florin et al., 1980; Rapson et al., 1980; Pagano et al., 1983; Haworth et al., 1983; Pagano et al., 1988; Wild et al., 1983; Westinghouse Electric Corporation, 1984; Heck et al., 1989; Gomes-Carneiro et al., 1998).

Eucalyptol [FL-no: 03.001] was tested in assays for sister chromatid exchange in Chinese hamster ovary cells *in vitro* (Galloway et al., 1987a; Sasaki et al., 1989). A statistically significant increase ($p < 0.05$) in the incidence of sister chromatid exchanges in the absence of metabolic activation was reported at high concentrations (200–500 µg/ml) that induced cell cycle delay (Galloway et al., 1987a). This finding was, however, not confirmed in a subsequent study that also used eucalyptol at concentrations that extended into the toxic range (Sasaki et al., 1989), nor was any increased incidence of sister chromatid exchange found in the presence of metabolic activation (Galloway et al., 1987a). In an assay for sister chromatid exchange in human lymphocytes *in vitro*, anisole [FL-no: 04.032) did not induce sister chromatid exchange at concentrations of up to 2 mmol/l (216 µg/ml) (Jansson et al., 1988).

Eucalyptol [FL-no: 03.001] did not induce chromosomal aberrations in Chinese hamster ovary cells at concentrations ranging from 479 to 663 µg/ml without metabolic activation, and from 630 to 810 µg/ml with metabolic activation (Galloway et al., 1987a). Diphenyl ether [FL-no: 04.035], at concentrations of 5 to 5000 µg/ml, did not induce chromosomal aberrations in Chinese hamster ovary cells with or without metabolic activation (San Sebastian, 1989b).

In an abstract for a preliminary screening study that was not published, 1-methoxy-4-methylbenzene [FL-no: 04.015] was tested in an assay for unscheduled DNA synthesis *in vitro* using hepatocytes isolated from adult male Fischer or Sprague-Dawley rats. Positive responses were reported for *p*-methylanisole, but only at cytotoxic concentrations (188 µg/ml; relative survival, 60 –78 %). At lower non-cytotoxic concentrations (5–100 µg/ml), there was no evidence of unscheduled DNA synthesis (Heck et al., 1989). Furthermore, incubation of the related substance *p*-propylanisole [FL-no: 04.039] with rat hepatocytes showed no evidence of unscheduled DNA synthesis (Howes et al., 1990). Diphenyl ether gave negative results in two separate assays for unscheduled DNA synthesis in rat hepatocytes *in vitro* at concentrations ranging from 0.5 to 100 µg/ml (Bakke and Mirsalis, 1987) and from 0.1 to 1000 µg/ml (Farr, 1987a).

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

In an assay for DNA repair in *Bacillus subtilis* H17 and M45 (rec assay), eucalyptol gave negative results at concentrations ranging from 18 to 20 000 mg/disc (Oda et al., 1979; Yoo, 1986).

In vivo

In an assay for micronucleus formation in bone marrow cells, male and female NMRI mice received single injections of *p*-propylanisole [FL-no: 04.039] at a dose of 750, 1125, or 1500 mg/kg bw in olive oil. The mice were killed 30 h after injection. Results were expressed as mean number of micronucleated polychromatic erythrocytes per 1000 polychromated erythrocytes. There was no evidence of an increase in the incidence of micronucleated polychromatic erythrocytes at any of the concentrations of *p*-propylanisole tested when compared with the values for controls (Wild et al., 1983).

Assays for micronucleus formation were also performed with four other aromatic ethers. There was no evidence of an increase in the frequency of micronucleated polychromatic erythrocytes reported when male and female NMRI mice were given intraperitoneal injections of *m*-dimethoxybenzene [FL-no: 04.016] at up to 1382 mg/kg bw, dibenzyl ether [FL-no: 03.004] at up to 1000 mg/kg bw, beta-naphthyl ethyl ether [FL-no: 04.033] at up to 861 mg/kg bw, or beta-naphthyl isobutyl ether [FL-no: 04.054] at up to 2000 mg/kg bw (Wild et al., 1983).

Assays for sex-linked recessive lethal mutations in *Drosophila melanogaster* were performed using 5 mmol/l of p-propylanisole [FL-no: 04.039], 25 mmol/l of *m*-dimethoxybenzene [FL-no: 04.016], 10 mmol/l of dibenzyl ether [FL-no: 03.004], 25 mmol/l of *beta*-naphthyl ethyl ether [FL-no: 04.033], or 25 mmol/l of *beta*-naphthyl isobutyl ether [FL-no: 04.054] (Wild et al., 1983). None of these substances was reported to give positive results in this assay (Wild et al., 1983).

In one of four assays with *p*-propylanisole [FL-no: 04.039], the frequency of sex-linked recessive lethal mutations was significantly increased ($p \leq 0.01$), a result that was not confirmed when the assay was repeated three times at the same test concentration (5 mmol/l). For *beta*-naphthyl isobutyl ether [FL-no: 04.054], a slight increase in sex-linked recessive lethal mutations "with a borderline significance of $p = 0.05$" was reported only in the second of three broods analysed, which the authors concluded to be of questionable relevance. The "borderline" significance was due to the abnormally low frequency of sex-linked recessive lethal mutations in the corresponding control brood for the second brood (0.19 %) compared with the values for controls (0.23 % and 0.29 %) for the other two broods.

Conclusion on genotoxicity

The Committee concluded that there was no confirmed evidence of genotoxicity for any of the aliphatic or aromatic ethers used as flavouring agents

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

3.2. Genotoxicity Studies - Text Taken[5] from EFSA FGE.23Rev2 (EFSA, 2010ac)

In vitro / in vivo

There are only four genotoxicity studies carried out on the candidate substances 1,2,3-trimethoxybenzene [FL-no: 04.084] and vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248]. These studies provided negative results but are of limited value. There have been a number of studies carried out on the supporting substances and these generally show that there is no cause for

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

concern regarding their genotoxicity. Two *in vitro* studies produced positive results; these studies are described in greater detail below. None of the *in vivo* tests showed positive results.

One of the *in vitro* genotoxicity studies (Heck et al., 1989) gave a positive result for the supporting substance 1-methoxy-4-methylbenzene [FL-no: 04.015] at a concentration of 188 microgram/ml. This study was an unscheduled DNA synthesis study. The test was carried out twice, but significant differences were seen between the initial results and the repeat assay and there was no explanation why these two results may have been different. Therefore, no definite conclusions could be drawn.

A positive result was seen in a sister chromatid exchange study on the supporting substance 1,8-cineole [FL-no: 03.001] (Galloway et al., 1987). This study was only positive without S9 activation and at levels of 1,8-cineole of 200 and 500 micrograms/ml which induced cell cycle delay and therefore were cytotoxic. There are several other genotoxicity tests on this substance, including another sister chromatid exchange study (although the concentrations of test substance were much lower in this study), that have given negative results. In the light of these results in several genotoxicity studies at gene and chromosomal level the positive result in the sister chromatid exchange assay by Galloway (Galloway et al., 1987) is considered not to be of relevance for the overall evaluation. It is therefore concluded that 1,8-cineole is not genotoxic.

Conclusion on genotoxicity

In summary the Panel concluded that the genotoxicity data available do not preclude the evaluation of the candidate substances through the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA see Table 2.2 and 2.3.

3.3. EFSA Considerations

The Panel concluded that the data available do not preclude evaluation of the 30 JECFA evaluated aliphatic and aromatic ethers through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 30 Aliphatic and Aromatic Ethers by the JECFA (JECFA, 2004b; JECFA, 2006a)

According to the JECFA nine of the substances belong to structural class I, 12 to structural class II and nine to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded 27 aliphatic and aromatic ethers at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and concluded that the intakes for the substances are below the thresholds for their structural classes I, II and III (step A3).

Three substances [FL-no: 03.001, 03.004 and 04.039] were concluded at step A5 – i.e. the intakes are above the thresholds for their structural classes, the substances are not endogenous, but for these three substances a NOAEL were found from which adequate margins of safety to the estimated intake of the substances [FL-no: 03.001, 03.004 and 04.039] could be calculated.

In conclusion, the JECFA evaluated all 30 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 30 aliphatic and aromatic ethers are summarised in Table 3.1: Summary of Safety Evaluation of 30 Aliphatic and Aromatic Ethers (JECFA, 2004b; JECFA, 2006a).

4.2. Application of the Procedure to 19 Aliphatic, Alicyclic and Aromatic Ethers Including Anisole Derivatives from Chemical Groups 15, 16, 22, 26 and 30 by EFSA (EFSA, 2010ac)

Nineteen substances were evaluated in FGE.23Rev2. Two substances are classified into structural class I, seven substances into structural class II and 10 substances into structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The 19 substances were all concluded at step A3 – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes are below the thresholds for the structural classes (step A3).

In conclusion the Panel evaluated all 19 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The stepwise evaluations of the 19 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA/ FGE.23Rev2).

4.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA for all 30 substances in the group of aliphatic and aromatic ethers.

Accordingly, these 30 substances do not pose a safety concern when used at estimated levels of intake as flavouring substances, based on the MSDI approach.

5. Conclusion

The Panel concluded that all the 30 substances in the JECFA flavouring group of aliphatic and aromatic ethers are structurally related to the group of 19 aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30 evaluated by EFSA in the Flavouring Group Evaluation 23, Revision 2 (FGE.23Rev2).

The Panel agrees with the application of the Procedure as performed by the JECFA for the 30 substances considered in this FGE.

For all 30 substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment to finalise the evaluation.

In order to determine whether the conclusion for the 30 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for 28 of the 30 JECFA evaluated substances. For two substances [FL-no: 13.037 and 13.072], have the composition of the mixture of diastereoisomers not been specified sufficiently. Thus, for the two substances [FL-no: 13.037 and 13.072], the Panel has reservations (information on stereoisomeric composition is missing). For the remaining 28 of the 30 JECFA evaluated aliphatic and aromatic ethers [FL-no: 03.001, 03.003, 03.004, 03.005, 03.006, 03.007, 03.010, 03.019, 04.014, 04.015, 04.016, 04.032, 04.033, 04.034, 04.035, 04.038, 04.039, 04.040, 04.043, 04.054, 04.062, 04.063, 04.074, 13.088, 13.094, 13.098, 13.165 and 16.088] 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

Table 1:Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2003b; JECFA, 2005b)

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of aliphatic and aromatic ethers (JECFA, 2003b; JECFA, 2005b)

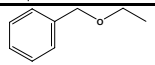
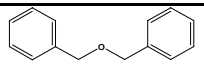
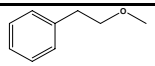
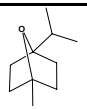
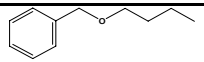
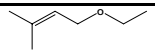
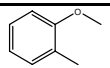
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
03.001 1234	1,8-Cineole		2465 182 470-82-6	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Soluble	176-177 IR 98 %	1.454-1.460 0.921-0.924	
03.003 1252	Benzyl ethyl ether		2144 521 539-30-0	Liquid $C_9H_{12}O$ 136.19	Insoluble Soluble	186-187 NMR 98 %	1.493-1.498 0.947-0.951 (20°)	
03.004 1256	Dibenzyl ether		2371 11856 103-50-4	Liquid $C_{14}H_{14}O$ 198.27	Insoluble Soluble	295-298 IR 99 %	1.558-1.563 1.040-1.045	
03.005 1231	2-Butyl ethyl ether		3131 10911 2679-87-0	Liquid $C_6H_{14}O$ 102.18	Insoluble Soluble	81 NMR 99 %	1.378-1.383 0.748-0.753 (20°)	Racemate (EFFA, 2010a)
03.006 1254	2-Methoxyethyl benzene		3198 11812 3558-60-9	Liquid $C_9H_{12}O$ 136.19	Insoluble Soluble	185-187 NMR 99 %	1.497-1.501 0.945-0.951	
03.007 1233	1,4-Cineole		3658 11225 470-67-7	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Soluble	172-174 NMR 75 %	1.449-1.456 0.898-0.902	1,4-Cineole (75%), secondary component 1,8-cineole (20-25%) (EFFA, 2010a)
03.010 1253	Benzyl butyl ether		2139 520 588-67-0	Liquid $C_{11}H_{16}O$ 164.25	Insoluble Soluble	220-221 NMR 92.6 %	1.480-1.485 0.928-0.933 (10°)	Minimum assay value is (92.6%), secondary component Benzyl alcohol (2-5%) (EFFA, 2010a)
03.019 1232	Prenyl ethyl ether		3777 22094-00-4	Liquid $C_7H_{14}O$ 114.19	Insoluble Soluble	64-66 (208 hPa) IR NMR MS 99.4 %	1.416-1.422 0.797-0.802	
04.014 1242	1-Methoxy-2-methylbenzene		2680 187 578-58-5	Liquid $C_8H_{10}O$ 122.17	Insoluble Soluble	172 - NMR 99 %	1.518-1.522 (15.3°) 0.983-0.986 (15.5°)	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of aliphatic and aromatic ethers (JECFA, 2003b; JECFA, 2005b)

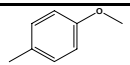
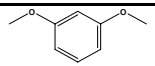
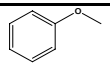
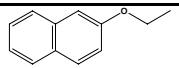
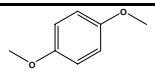
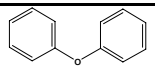
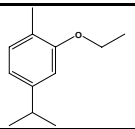
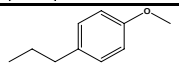
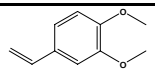
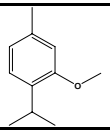
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
04.015 1243	1-Methoxy-4-methylbenzene		2681 188 104-93-8	Liquid $C_8H_{10}O$ 122.17	Insoluble Soluble	174 - IR 99 %	1.510-1.513 0.996-1.004	
04.016 1249	1,3-Dimethoxybenzene		2385 189 151-10-0	Liquid $C_8H_{10}O_2$ 138.17	Slightly soluble Soluble	215-217.5 - NMR 97 %	1.521-1.527 1.053-1.057	
04.032 1241	Anisole		2097 2056 100-66-3	Liquid C_7H_8O 108.14	Insoluble Soluble	154 - IR 99 %	1.515-1.518 0.990-0.993	
04.033 1258	beta-Naphthyl ethyl ether		2768 2058 93-18-5	Solid $C_{12}H_{12}O$ 172.23	Insoluble Soluble	n.a. 37 NMR 99 %	n.a. n.a.	
04.034 1250	1,4-Dimethoxybenzene		2386 2059 150-78-7	Solid $C_8H_{10}O_2$ 138.17	Slightly soluble Soluble	n.a. 56-60 NMR 98 %	n.a. n.a.	
04.035 1255	Diphenyl ether		3667 2201 101-84-8	Solid $C_{12}H_{10}O$ 170.21	Insoluble Soluble	259 26.8 NMR 99 %	1.578-1.583 1.071-1.075	
04.038 1247	Carvacryl ethyl ether		2246 11840 4732-13-2	Liquid $C_{12}H_{18}O$ 178.28	Slightly soluble Soluble	235 - NMR 95 %	1.502-1.509 0.935-0.942	
04.039 1244	1-Methoxy-4-propylbenzene		2930 11835 104-45-0	Liquid $C_{10}H_{14}O$ 150.22	Slightly soluble Soluble	212-213 - IR 99 %	1.503-1.506 0.940-0.943	
04.040 1251	1,2-Dimethoxy-4-vinylbenzene		3138 11228 6380-23-0	Liquid $C_{10}H_{12}O_2$ 164.20	Slightly soluble Soluble	203-205 (26hPa) - NMR 99 %	1.520-1.526 1.006-1.012	
04.043 1246	1-Isopropyl-2-methoxy-4-methylbenzene		3436 11245 1076-56-8	Liquid $C_{11}H_{16}O$ 164.25	Insoluble Soluble	216 - NMR 98 %	1.504-1.508 0.936-0.940	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of aliphatic and aromatic ethers (JECFA, 2003b; JECFA, 2005b)

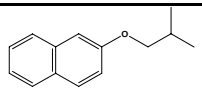
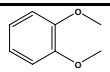
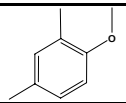
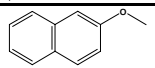
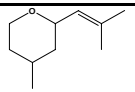
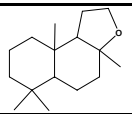
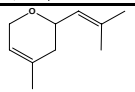
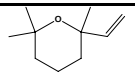
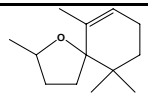
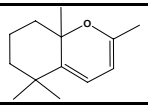
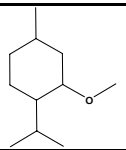
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
04.054 1259	Isobutyl beta-naphthyl ether		3719 11886 2173-57-1	Solid $C_{14}H_{16}O$ 200.28	Insoluble Soluble	n.a. 33 NMR 98 %	n.a. n.a.	
04.062 1248	1,2-Dimethoxybenzene		3799 10320 91-16-7	Liquid $C_8H_{10}O_2$ 138.17	Insoluble Soluble	206-207 IR NMR 98 %	1.533-1.536 1.082-1.086	
04.063 1245	1,3-Dimethyl-4-methoxybenzene		3828 - 6738-23-4	Liquid $C_9H_{12}O$ 136.20	Slightly soluble Soluble	193 - IR NMR MS 96.5 %	1.512-1.516 0.963-0.967	
04.074 1257	2-Methoxynaphthalene		- - 93-04-9	Solid $C_{11}H_{10}O$ 158.20	Insoluble Soluble	n.a. 73-75 NMR 99 %	n.a. n.a.	
13.037 1237	2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran		3236 2269 16409-43-1	Liquid $C_{10}H_{18}O$ 154.25	Slightly soluble Soluble	182 - NMR 99 %	1.453-1.457 0.873-0.877	Mixture of diastereoisomers (EFFA, 2010a).
13.072 1240	1,5,5,9-Tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane		3471 10514 3738-00-9	Solid $C_{16}H_{28}O$ 236.40	Insoluble Soluble	n.a. 75-85 NMR 96 %	n.a. n.a.	Mixture of diastereoisomers (EFFA, 2010a).
13.088 1235	3,6-Dihydro-4-methyl-2-(2-methylprop-1-en-1-yl)-2H-pyran		3661 - 1786-08-9	Liquid $C_{10}H_{16}O$ 152.24	Insoluble Soluble	68-72 (9 hPa) - IR NMR MS 97 %	1.472-1.478 0.900-0.908	Racemate (EFFA, 2010a).
13.094 1236	2,6,6-Trimethyl-2-vinyltetrahydropyran		3735 10976 7392-19-0	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Soluble	22 (3 hPa) - NMR 99 %	1.446-1.452 0.866-0.871	Racemate (EFFA, 2010a).
13.098 1238	Theaspirane		3774 10515 36431-72-8	Liquid $C_{13}H_{22}O$ 194.32	Insoluble Soluble	65 (1 hPa) - IR NMR MS 97 %	1.487-1.490 0.938-0.943	Racemate, and mixture of (Z)-isomer (55-58%), (E)-isomer (42-45%) and 5 other secondary components 8-13.5% (EFFA, 2010a).
13.165 1239	6,7,8,8a-Tetrahydro-2,5,5,8a-tetramethyl-5H-1-benzopyran		3822 - 5552-30-7	Liquid $C_{13}H_{20}O$ 192.3	Insoluble Soluble	240 - IR NMR 96.7 %	1.499-1.505 0.950-0.955	Racemate (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of aliphatic and aromatic ethers (JECFA, 2003b; JECFA, 2005b)

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
16.088 1415	l-Menthylmethylether		4054 - 1565-76-0	Liquid $C_{11}H_{22}O$ 170.30	Insoluble Soluble	81 (14 hPa) - IR NMR MS 99 %	1.441-1.447 0.856-0.862	

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.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (in vitro / in vivo) for 30 Aliphatic and Aromatic Ethers (JECFA, 2004b)

Table 2.1: Summary of Genotoxicity Data for 30 Aliphatic and Aromatic Ethers

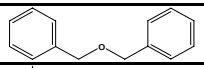
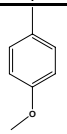
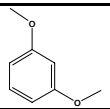
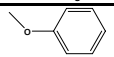
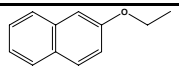
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
03.001 1234	1,8-Cineole		Reverse mutation	*S. typhimurium* TA102, TA100, TA98, TA97	250–2500 µg/plate	Negative[a]	(Gomes-Carneiro et al., 1998)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3.3–3333 µg/plate	Negative[a,b]	(Haworth et al., 1983)
			Sister chromatid exchange	Chinese hamster ovary cells	50–500 µg/ml[c] 600–800 µg/ml	Positive[d] Negative[e]	(Galloway et al., 1987a)
			Sister chromatid exchange	Chinese hamster ovary cells CHO K-1	10, 33.3 and 100 µmol/l (1.5, 5.1 and 15.4 µg/ml)[f]	Negative[d]	(Sasaki et al., 1989)
			Chromosomal aberrations	Chinese hamster ovary cells	479–663 µg/ml 630–810 µg/ml	Negative[d] Negative[e]	(Galloway et al., 1987a)
			DNA repair	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	18 µg/disk	Negative[g]	(Oda et al., 1979)
			DNA repair	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	≤ 20 µl/disk (20000 µg /disk)[h]	Negative[g]	(Yoo, 1986)
03.004 1256	Dibenzyl ether		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤ 3.6 mg/plate	Negative[a]	(Wild et al., 1983)
04.015 1243	1-Methoxy-4-methylbenzene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (367µg/ plate)[j]	Negative[a]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	50000 µg/plate	Negative[a]	(Heck et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	188 µg/ml	Positive	(Heck et al., 1989)
04.016 1249	1,3-Dimethoxybenzene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤ 3.6 mg/plate	Negative[a]	(Wild et al., 1983)
04.032 1241	Anisole		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537	3 µmol/plate (324 µg /plate)[i]	Negative[a]	(Florin et al., 1980)
			Sister chromatid exchange	Human lymphocytes	0–2.0 mol/l (0– 216 µg/ml)[i]	Negative	(Jansson et al., 1988)
04.033 1258	beta-Naphthyl ethyl ether		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤ 3.6 mg/plate	Negative[a]	(Wild et al., 1983)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 mmol/plate (517µg/ plate)[p]	Negative[a]	(Florin et al., 1980)

Table 2.1: Summary of Genotoxicity Data for 30 Aliphatic and Aromatic Ethers

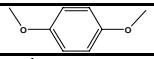
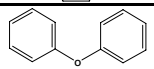
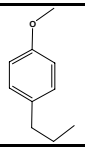
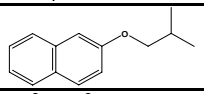
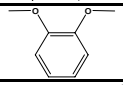
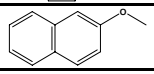
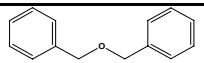
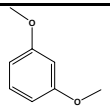
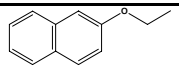
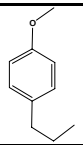
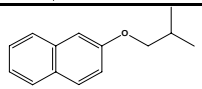
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
04.034 1250	1,4-Dimethoxybenzene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10–900 µg/plate	Negative[a,b]	(Haworth et al., 1983)
04.035 1255	Diphenyl ether		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (511 µg/ plate)[l]	Negative[a]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1532, TA1535, TA1537, TA1538, TA2636	0.1–500 µg/plate	Negative[a]	(Pagano et al., 1983)
			Reverse mutation	*S. typhimurium* TA100, TA97, TA98, TA102	≤ Cytotoxic concentrations (≥ 10^{-2} mmol/l or 17 µg/ml)[l]	Negative[a]	(Pagano et al., 1988)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3.3–333.3 µg/plate	Negative[a,b]	(Pagano et al., 1983)
			Reverse mutation	*S. typhimurium* TA100, TA1538, TA98, TA1537, TA1535	1–10 000 µg/plate	Negative[a]	(Clark et al., 1979)
			Reverse mutation	*S. typhimurium* TA1535, TA100, TA1538, TA98, TA1537, TA1978	5 and 10 µl/plate (5000 and 11 000 µg/plate)[m]	Negaitve[a]	(Westinghouse Electric Corporation, 1984)
			Mutations	*Saccharomyces cerevisiae* D7	Up to 1 mmol/l (170 µg/ml)[l]	Negative[a]	(Pagano et al., 1983)
			Chromosomal aberrations	Chinese hamster ovary cells	5–5000 µg/ml	Negative[a]	(San Sebastian, 1989b)
			Unscheduled DNA synthesis	Rat hepatocytes	0.5–100 µg/ml	Negative	(Bakke and Mirsalis, 1987)
			Unscheduled DNA synthesis	Rat hepatocytes	0.1–1000 µg/ml[n]	Negative	(Farr, 1987a)
04.039 1244	1-Methoxy-4-propylbenzene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤750 µg/plate	Negative[a]	(Wild et al., 1983)
			Unscheduled DNA synthesis	Rat hepatocytes	≥5 x 10^{-3} mol/l (751µg/ml)[k] and above	Negative	(Howes et al., 1990)
04.054 1259	Isobutyl beta-naphthyl ether		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	≤1 mg/plate	Negative[a]	(Wild et al., 1983)
04.062 1248	1,2-Dimethoxybenzene		Reverse mutation	*S. typhimurium* TA100	0.1, 1, 10, 100 and 1000 µg/ plate	Negative	(Rapson et al., 1980)
04.074 1257	2-Methoxynaphthalene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate (475 µg/ plate)[o]	Negative[a]	(Florin et al., 1980)
In vivo							
03.004 1256	Dibenzyl ether		Micronucleus formation	Mice	400, 700 or 1000 mg/kgbw[t]	Negative[q]	(Wild et al., 1983)
			Sex-linked recessive lethal mutation	*Drosophila melanogaster*	10 mmol/l (1983µg/ml)[u]	Negative	(Wild et al., 1983)

Table 2.1: Summary of Genotoxicity Data for 30 Aliphatic and Aromatic Ethers

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
04.016 1249	1,3-Dimethoxybenzene		Micronucleus formation	Mice	558, 966, or 1382 mg/kgbw	Negative[q]	(Wild et al., 1983)
			Sex-linked recessive lethal mutation	*Drosophila melanogaster*	25 mmol/l (3454 µg/ml)[s]	Negative	(Wild et al., 1983)
04.033 1258	beta-Naphthyl ethyl ether		Micronucleus formation	Mice	344, 603, 861 mg/kgbw	Negative[q]	(Wild et al., 1983)
			Sex-linked recessive lethal mutation	*Drosophila melanogaster*	25 mmol/l (4,306 µg/ml)[p]	Negative	(Wild et al., 1983)
04.039 1244	1-Methoxy-4-propylbenzene		Micronucleus formation	Mice	750, 1125, 1500mg/kg	Negative[q]	(Wild et al., 1983)
			Sex-linked recessive lethal mutation	*Drosophila melanogaster*	5 mmol/l (751µg/ml)[k]	Negative[r]	(Wild et al., 1983)
04.054 1259	Isobutyl beta-naphthyl ether		Micronucleus formation	Mice	800, 1,400, or 2000 mg/kgbw	Negative[q]	(Wild et al., 1983)
			Sex-linked recessive lethal mutation	*Drosophila melanogaster*	25 mmol/l	Negative[v]	(Wild et al., 1983)

a With or without metabolic activation.
b Pre-incubation method.
c Lowest dose to give a significant increase in sister chromatid exchange: Trial I—500 µg/ml, Trial II—200 µg/ml.
d Without metabolic activation.
e With metabolic activation.
f Calculated using relative molecular mass of eucalyptol = 154.25.
g Foreign language article, data available from English abstract and/or tables.
h Calculated using density of eucalyptol = 0.921–0.924 (Food Chemical Codex, 1996).
i Calculated using relative molecular mass of anisole = 108.14.
j Calculated using relative molecular mass of p-methylanisole = 122.17.
k Calculated using relative molecular mass of p-propylanisole = 150.22.
l Calculated using relative molecular mass of diphenyl ether = 170.21.
m Calculated using density of diphenyl ether = 1.07 (Arctander, 1969).
n These values are for a mixture containing 73.5% diphenyl ether and 26.5% biphenyl.
o Calculated using relative molecular mass of _-naphthyl methyl ether = 158.2.
p Calculated using relative molecular mass of _-naphthyl ethyl ether = 172.23.
q Administered via intraperitoneal injection.
r In one of the four tests using I-propylanisole, high frequencies of sex-linked recessive lethal mutation were observed in two broods, which were significantly ($p \leq 0.01$) above the control value. However, the authors noted that four doubles (two lethal mutations from one male) were observed in the test, and due to the lack of effects seen in the other three tests, the doubles were considered pre-existing and of spontaneous origin (Wild et al., 1983).
s Calculated using relative molecular mass of m-dimethoxybenzene = 138.17.
t Administered twice within a 24-hoursperiod.
u Calculated using relative molecular mass of dibenzyl ether= 198.27.
v A slight increase "with a borderline significance of $p = 0.05$" in frequencies of sex-linked recessive lethal mutations was reported in the second brood of three, which was considered of questionable relevance and not a positive result (Wild et al., 1983). The "borderline" significance reported ($p = 0.05$) appears to be due to the abnormally low frequency of sex-linked recessive lethal mutations in the corresponding control brood (control brood II: 34/17734 or 0.19%) when compared to the control groups of the other two broods (control brood I: 42/18188 or 0.23 % and control brood III: 50/16980 or 0.29 %).

Table 2.2: Genotoxicity Data (in vitro) EFSA / FGE.23Rev2

Substances listed in brackets are the JECFA evaluated supporting substances in FGE.23Rev2

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Anisole [04.032])	Ames reverse mutation assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	2.
	Sister chromatid exchange	Human lymphocytes	2 mM	Negative (-S9 only)	(Jansson et al., 1988)	2.
(1-Methoxy-4-methylbenzene [04.015])	Ames reverse mutation assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	Published Non-GLP study. Limited report of study details. Validity of the study cannot be evaluated.
	Ames reverse mutation assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	50 mg/plate	Negative (+/- S9)	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis	Rat hepatocytes	188 µg/ml	Positive	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(1,2-Dimethoxybenzene [04.062])	Ames reverse mutation assay	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	2.
(1,3-Dimethoxybenzen [04.016])	Ames reverse mutation assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3.6 mg/plate	Negative (+/- S9)	(Wild et al., 1983)	2.
(1,4-Dimethoxybenzene [04.034])	Ames reverse mutation assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	900 µg/plate	Negative (+/- S9)	(Haworth et al., 1983)	2.
	Ames reverse mutation assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 ng/plate	Negative (+/- S9)	(CIT)	2.
1,2,3-Trimethoxybenzene [04.084]	Ames reverse mutation assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	Tested quantitatively with TA100. Published non-GLP study. Limited report of study details. No results reported. Validity of the study cannot be evaluated.
	SOS Chromotest	*E. coli* PQ37	NR	Negative	(Ohshima et al., 1989)	Study assessing the SOS-inducing potency of a range of phenols after nitrosation *in vitro* in the absence of metabolic activation. The result for 1,2,3-trimethoxybenzene was negative.
(1,8-Cineole [03.001])	Ames reverse mutation assay	*S. typhimurium* TA97; TA98; TA100; TA102	2500 µg/plate	Negative (+/- S9)	(Gomes-Carneiro et al., 1998)	Published non-GLP study. Fairly detailed description of study details and results, generally follows OECD guidelines. Study considered valid.
	Ames reverse mutation assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative (+/- S9)	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(1,8-Cineole [03.001]) cont.	Sister chromatid exchange	Chinese hamster ovary cells	500 µg/ml 800 µg/ml	Positive (-S9) Negative (+S9)	(Galloway et al., 1987a)	Lowest dose to give a significant increase in SCE: Trial I – 500 µg/ml; Trial II – 200 µg/ml. Published non-GLP study. Doses were selected based on preliminary assay. Some details of results are not reported. Test was positive only without activation and at doses that induced cell cycle delay.
	Sister chromatid exchange	Chinese hamster ovary CHO K-1 cells	100 µM	Negative (-S9 only)	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs.
	Chromosomal aberration assay	Chinese hamster ovary cells	663 µg/ml 810 µg/ml	Negative (+/- S9)	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid. No aberration induction was detected even after extending the incubation time without S9 to 20 hours.
	Rec assay	*B. subtilis* H17 (rec+) and M45 (rec-)	18 µg/disk	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated. The SOS chromotest is not considered predictive for genotoxicity.
	Rec assay	*B. subtilis* H17 (rec+) and M45 (rec-)	20 µl/disk (20,000µg/disk)	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. The SOS chromotest is not considered predictive for genotoxicity.
(1-Methoxy-4-propylbenzene 04.039])	Ames reverse mutation assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	750 µg/plate	Negative (+/- S9)	(Wild et al., 1983)	2.
	Unscheduled DNA synthesis	Rat hepatocytes	$5x10^{-3}$ M	Negative	(Howes et al., 1990)	2.
(2-Methoxynaphthalene [04.074])	Ames reverse mutation assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	2.
(beta-Naphthyl ethyl ether [04.033])	Ames reverse mutation assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3.6 mg/plate	Negative (+/- S9)	(Wild et al., 1983)	2.
	Ames reverse mutation assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	2.
(Isobutyl beta-naphthyl ether [04.054])	Ames reverse mutation assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1 mg/plate	Negative (+/- S9)	(Wild et al., 1983)	2.
(2-Phenoxyethyl isobutyrate [09.487])	Ames reverse mutation assay	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3600 µg/plate	Negative (+/- S9)	(Wild et al., 1983)	3.
(Phenoxyacetic acid [08.049])	Mutagenicity assay	*S. cerevisiae* D7tsl	16 mM	Negative (- S9)	(Venkov et al., 2000)	3.
(Alpha-terpineol [02.014])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10000 µg/plate	Negative	(Heck et al., 1989)	4.
	Ames test	*S. typhimurium* TA97a; TA98; TA100; TA102	2500 µg/plate	Negative[1]	(Gomes-Carneiro et al., 1998)	4.
(Alpha-terpineol [02.014]) cont.	Ames test	*S. typhimurium* TA98; TA100; TA1535;	1000 µg/plate	Negative (+/- S9)	(National Cancer	4.

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA1537; TA1538			Institute, 1983)	
	Spot test	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µg/plate (463 µg/plate)	Negative (+/- S9)	(Florin et al., 1980)	4.
	Mammalian cell mutation	Mouse Lymphoma L5178Y TK +/-	0.5 µl/ml (467µg/ml) 0.75µl/ml (700 µg/ml)	Negative (- S9) Negative (+S9)	(Kirby et al., 1984)	4.
	Mammalian cell mutation	Mouse Lymphoma L5178Y TK +/-	300 nl/ml (280 µg/ml) 250 nl/ml (233 µg/ml)	Negative (+/- S9)	(Heck et al., 1989)	4.
	Rec assay	*S. cerevisiae*	NR	Negative	(Oda et al., 1979)	4.
(Terpineol acetate [09.830])	Rec assay	*B. subtilis* H17; M45	19 µg	Negative	(Oda et al., 1979)	4.
Vanillin 3-(l-menthoxy)propane-1,2-diol acetal [02.248]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative (+/- S9)	(Kajiura, 1996b)	The study is not completely in accordance with OECD guidelines (471): no confirmation of negative findings in an independent experiment and only two plates pr concentration.
	Ames test	*E. coli* WP2 uvrA	Up to 5000 µg/plate	Negative (+/- S9)	(Kajiura, 1996b)	The study is not completely in accordance with OECD guidelines (471): no confirmation of negative findings in an independent experiment and only two plates pr concentration.

1 A slight but dose-related response was noted with TA102 with and without the use of metabolic activation.
2 Summarised by JECFA 61st meeting (JECFA, 2004b).
3 Summarised by JECFA 59th meeting (JECFA, 2003a).
4 Summarised by JECFA 51st meeting (JECFA, 1999a .

Table 2.3: Genotoxicity Data (in vivo) for EFSA / FGE.23Rev2

Substances listed in brackets are the JECFA evaluated supporting substances in FGE.23Rev2

Table 2.3: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(1,3-Dimethoxybenzene [04.016])	*In vivo* Micronucleus test	Mouse	Intraperitoneal injection	1382 mg/kg bw	Negative	(Wild et al., 1983)	1
	In vivo Sex- linked recessive lethal mutation assay	*D. melanogaster*		25 mM	Negative	(Wild et al., 1983)	1
(1,4-Dimethoxybenzene [04.034])	*In vivo* Micronucleus test	Mouse	Oral gavage	2000 mg/kg bw	Negative	(Hoechst, 1996)	1
(1-Methoxy-4-propylbenzene [04.039])	*In vivo* Micronucleus test	Mouse	Intraperitoneal injection	1500 mg/kg	Negative	(Wild et al., 1983)	1
	In vivo Sex- linked recessive lethal mutation assay	*D. melanogaster*	(751 µg/ml)	5 mM	Negative (+/- S9)	(Wild et al., 1983)	1
(beta-Naphthyl ethyl ether [04.033])	*In vivo* Micronucleus test	Mouse	Intraperitoneal injection	861 mg/kg bw	Negative	(Wild et al., 1983)	1
	In vivo Sex- linked recessive lethal mutation assay	*D. melanogaster*		25 mM	Negative	(Wild et al., 1983)	1
(Isobutyl beta-naphthyl ether [04.054])	*In vivo* Micronucleus test	Mouse	Intraperitoneal injection	2000 mg/kg bw	Negative	(Wild et al., 1983)	1
	In vivo Sex- linked recessive lethal mutation assay	*D. melanogaster*		25 mM	Negative	(Wild et al., 1983)	1
(2-Phenoxyethyl isobutyrate [09.487])	*In vivo* Micronucleus formation assay	Mouse bone marrow cells	IP injection	1875 mg/kg/bw	Negative	(Wild et al., 1983)	2
	In vivo Sex-linked recessive mutation	D. melanogaster		10 mM	Negative	(Wild et al., 1983)	2

1 Summarised by JECFA 61st meeting (JECFA, 2004b)
2 Summarised by JECFA 59th meeting (JECFA, 2003a)

TABLE 3.1: SUMMARY OF SAFETY EVALUATION OF ALIPHATIC AND AROMATIC ETHERS (JECFA, 2004B; JECFA, 2006A)

Table 3.1: Summary of Safety Evaluation of Aliphatic and Aromatic Ethers *(JECFA, 2004b; JECFA, 2006d)*

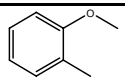
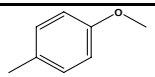
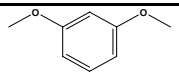
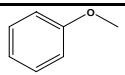
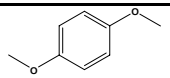
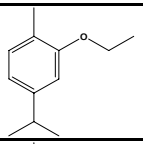
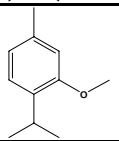
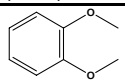
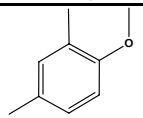
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
04.014 1242	1-Methoxy-2-methylbenzene		2.4 0.06	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.015 1243	1-Methoxy-4-methylbenzene		0.49 15	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.016 1249	1,3-Dimethoxybenzene		4.6 2	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.032 1241	Anisole		0.024 0.01	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.034 1250	1,4-Dimethoxybenzene		15 7	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.038 1247	Carvacryl ethyl ether		0.085 0.02	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.043 1246	1-Isopropyl-2-methoxy-4-methylbenzene		1.7 0.1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.062 1248	1,2-Dimethoxybenzene		1.6 20	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
04.063 1245	1,3-Dimethyl-4-methoxybenzene		0.12 0.2	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of Aliphatic and Aromatic Ethers *(JECFA, 2004b; JECFA, 2006d)*

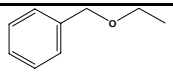
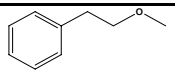
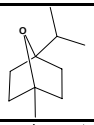
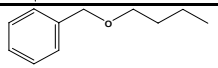
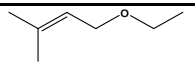
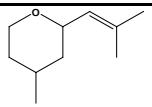
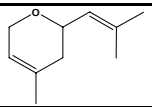
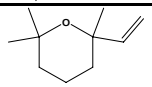
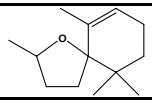
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
03.003 1252	Benzyl ethyl ether		0.0024 2	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
03.005 1231	2-Butyl ethyl ether		6.9 0.3	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
03.006 1254	2-Methoxyethyl benzene		26 0.01	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
03.007 1233	1,4-Cineole		3.9 146	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	
03.010 1253	Benzyl butyl ether		0.012 0.02	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	According to JECFA: Min. assay value is "92.6" and secondary components "Benzyl alcohol". No safety concern at the estimated level of intake based on the MSDI approach.
03.019 1232	Prenyl ethyl ether		0.73 2	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
13.037 1237	2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran		3.8 0.2	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	Composition of stereoisomeric mixture to be specified. No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
13.088 1235	3,6-Dihydro-4-methyl-2-(2-methylprop-1-en-1-yl)-2H-pyran		0.85 0.7	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
13.094 1236	2,6,6-Trimethyl-2-vinyltetrahydropyran		0.012 8	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
13.098 1238	Theaspirane		1.7 0.1	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake 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 Aliphatic and Aromatic Ethers *(JECFA, 2004b; JECFA, 2006d)*

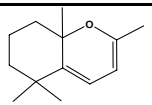
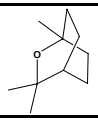
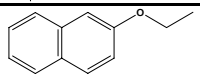
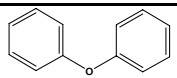
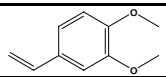
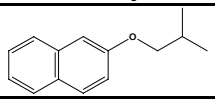
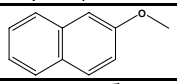
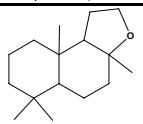
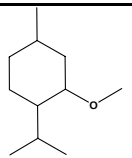
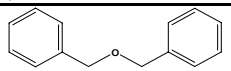
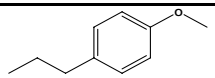
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.165 1239	6,7,8,8a-Tetrahydro-2,5,5,8a-tetramethyl-5H-1-benzopyran		0.14 2	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	Racemate. No safety concern at the estimated level of intake based on the MSDI approach.
03.001 1234	1,8-Cineole		1200 1954	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.033 1258	beta-Naphthyl ethyl ether		43 4	Class III A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
04.035 1255	Diphenyl ether		12 5	Class III A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.040 1251	1,2-Dimethoxy-4-vinylbenzene		0.012 0.01	Class III A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
04.054 1259	Isobutyl beta-naphthyl ether		1.2 2	Class III A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
04.074 1257	2-Methoxynaphthalene		3.5 0.01	Class III A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
13.072 1240	1,5,5,9-Tetramethyl-13-oxatricyclo [8.3.0.0.(4.9)]tridecane		1.2 0.1	Class III A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	Composition of stereoisomeric mixture to be specified No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
16.088 1415	l-Menthylmethylether		1.2 53	Class III A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach	CASrn refers to the (1S,2R,4R) isomer. The name specify the isomer (1S,2R,4R). No safety concern at the estimated level of intake based on the MSDI approach.
03.004 1256	Dibenzyl ether		0.49 241	Class III A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake 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 Aliphatic and Aromatic Ethers *(JECFA, 2004b; JECFA, 2006d)*

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
04.039 1244	1-Methoxy-4-propylbenzene		20 114	Class III A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.

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

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.23Rev2)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA, 2010ac)

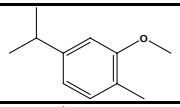
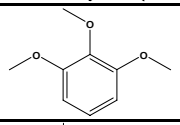
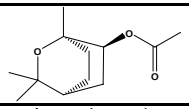
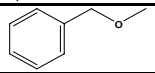
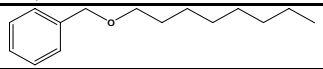
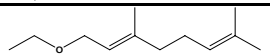
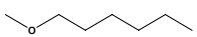
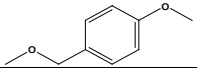
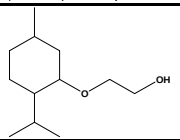
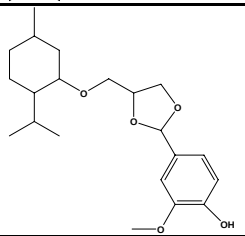
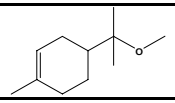
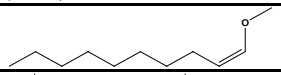
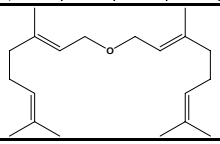
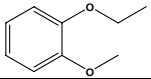
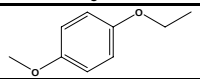
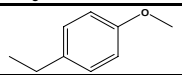
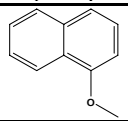
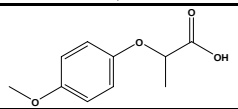
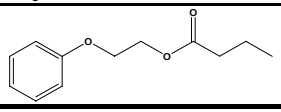
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]
04.059	Carvacryl methyl ether		1.2	Class I A3: Intake below threshold	4)	6)
04.084	1,2,3-Trimethoxybenzene		0.012	Class I A3: Intake below threshold	4)	6)
03.008	2-Acetoxy-1,8-cineole		0.037	Class II A3: Intake below threshold	4)	6)
03.011	Benzyl methyl ether		1.9	Class II A3: Intake below threshold	4)	6)
03.012	Benzyl octyl ether		0.24	Class II A3: Intake below threshold	4)	6)
03.015	Ethyl geranyl ether		0.012	Class II A3: Intake below threshold	4)	6)
03.016	Hexyl methyl ether		0.012	Class II A3: Intake below threshold	4)	6)
04.079	Methyl-4-methoxybenzyl ether		0.61	Class II A3: Intake below threshold	4)	6)
02.247 1853	l-Menthoxyethanol		15	Class III A3: Intake below threshold	4)	6)
02.248 1879	Vanillin 3-(l-menthoxy)propane-1,2-diol acetal		0.61	Class III A3: Intake below threshold	4)	6)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA, 2010ac)

FL-no JECFA-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)]
03.020	alpha-Terpinyl methyl ether		4.1	Class III A3: Intake below threshold	4)	6)
03.022 1802	1-Methoxy-1-decene		6.1	Class III A3: Intake below threshold	4)	7)
03.024	Digeranyl ether		49	Class II A3: Intake below threshold	4)	6)
04.067	1-Ethoxy-2-methoxybenzene		0.12	Class III A3: Intake below threshold	4)	6)
04.068	1-Ethoxy-4-methoxybenzene		0.67	Class III A3: Intake below threshold	4)	6)
04.069	1-Ethyl-4-methoxybenzene		0.073	Class III A3: Intake below threshold	4)	6)
04.075	1-Methoxynaphthalene		0.061	Class III A3: Intake below threshold	4)	6)
08.127	2-(4-Methoxyphenoxy)propionic acid		0.011	Class III A3: Intake below threshold	4)	6)
09.687	2-Phenoxyethyl butyrate		0.085	Class III A3: Intake below threshold	4)	6)

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

REFERENCES

Bakke JP and Mirsalis JC, 1987. Evaluation of the potential of diphenyl oxide to induce unscheduled DNA synthesis in primary rat hepatocyte cultures (final report) with attachments and cover sheet dated 060889. Monsanto Co. EPA Doc. 86-89000342, microfiche no. OTS0518139. Date 2/09/87. Unpublished report submitted by EFFA to FLAVIS Secretariat.

CIT (date not given) HOE 88.0890. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 150-78-7, EINECS Name 1,4-dimethoxybenzene. Section 1.0.1-7.1.

Clark CR, Marshall TC, Merickel BS, Sanchez A, Brownstein DG and Hobbs CH, 1979. Toxicological assessment of heat transfer fluids proposed for use in solar energy applications. Toxicol. Appl. Pharmacol. 51(3), 529-535.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

EC, 1996a. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC, 2000a. Commission Regulation 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. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.

EFFA, 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

EFSA, 2008ac. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 59: Consideration of aliphatic and aromatic ethers evaluated by JECFA (61st meeting) structurally related to aliphatic, alicyclic and aromatic ethers including anisole derivatives evaluated by EFSA in FGE.23 (2006) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 3 July 2007. EFSA-Q-2008-032K.

EFSA, 2008bg. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 23, Revision 1: Aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical group 15, 16 and 30 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 29 November 2006. EFSA-Q-2003-166a.

EFSA, 2010ac. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from Commission related to Flavouring Group Evaluation 23, revision 2 (FGE.23Rev2): Aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 29 September 2010. EFSA-Q-2009-00580.

EFSA, 2010aj. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from Commission related to Flavouring Group Evaluation 96 (FGE.96), addendum to FGE. 50, 51, 52, 53, 54, 56, 58, 59, 61, 62, 63, 64, 66, 68, 69, 70, 71, 73, 76, 77, 79, 80, 83, 84, 85, 86 and 87: Consideration of 102 flavouring substances considered by EFSA for which EU production volumes / anticipated production volumens have been submitted on request by DG SANCO (Commission Regulation (EC) No 1565/2000 of 18 July 2000).

Farr CH, 1987a. Range-finding teratology study in rats with Therminol VP-1. Monsanto Company. Study no. BD-86-379. June 22, 1987. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology. 18, 219-232.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA, Anderson B and Zeiger E, 1987a. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ. Mol. Mutag. 10(Suppl. 10), 1-175.

Gomes-Carneiro MR, Felzenszwalb I and Paumgartten FJ, 1998. Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay. Mutat. Res. 416, 129-136.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environ. Mutag.5 (Suppl. 1) 3-142.

Heck JD, Vollmuth TA, Cifone MA, Jagannath DR, Myhr B and Curren RD, 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1), 257-272.

Hoechst AG, 1996. Unveröffentl. Unters. (Ber.-Nr. 96.0064 vom 15.02.1996). Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 150-78-7, EINECS Name 1,4-dimethoxybenzene. Section 1.0.1-5.11.

Howes AJ, Chan VSW and Caldwell J, 1990. Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis in rat hepatocytes. Food Chem. Toxicol. 28(8), 537-542.

Jansson T, Curvall M, Hedin A and Enzell C, 1988. In vitro studies of the biological effects of cigarette smoke condensate. III. Induction of SCE by some phenolic and related constituents derived from cigarette smoke. Mutat. Res. 206, 17-24.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA, 2003b. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Committee of Food Additives 61st session. Rome, 10-19 June 2003. FAO Food and Nutrition paper 52 Add. 11.

JECFA, 2004b. Safety evaluation of certain food additives and contaminants. Sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 52. IPCS, WHO, Geneva.

JECFA, 2005b. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

JECFA, 2006a. Safety evaluation of certain food additives and contaminants. Sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.

JECFA, 2006c. Joint FAO/WHO Expert Committee on Food Additives. Sixty-seventh meeting. Rome, 20-29 June 2006, Summary and Conclusions. Issued 7 July 2006.

JECFA, 2006d. Safety evaluation of certain food additives and contaminants. Sixty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 56. IPCS, WHO, Geneva.

Kajiura Y, 1996b. Mutagenicity test of HOTACT 1MM {4-(l-menthoxymethyl)-2-(3'-methoxy-4'-hydroxyphenyl)-1,3-dioxolane}. Central Research Laboratory. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Kirby PE, Duglas-Tabor Y, Simmons RT, Voglezon RA, Rogers-Back AM, Brauninger RM, O'Keefe TR and Fernandez-Madrid AM, 1984. Mouse lymphoma mutagenesis assay with #70437 (alpha-terpineol). Short-term test program sponsored by The Division of Cancer Etiology, National Cancer Institute. Study no. ML-NCI#109. Unpublished data submitted by EFFA to FLAVIS Secretariat.

National Cancer Institute, 1983. Mutagenicity of G70437. alpha-Terpineol. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Oda Y, Hamono Y, Inoue K, Yamamoto H, Niihara T and Kunita N, 1979. [Mutagenicity of food flavors in bacteria]. Shokuhin. Eisei. Hen. 9, 177-181. (In Japanese)

Ohshima H, Friesen M, Malaveille C, Brouet I, Hautefeuille A and Bartsch H, 1989. Formation of direct-acting genotoxic substances in nitrosated smoked fish and meat products: Identification of simple phenolic precursors and phenyldiazonium ions as reactive products. Food Chem. Toxicol. 27(3), 193-203.

Pagano G, Esposito A, Giordano GG, Vamvakinos E, Quinto I, Bronzetti G, Bauer C, Corsi C, Nieri R and Ciajolo A, 1983. Genotoxicity and teratogenicity of diphenyl and diphenyl ether: A study of sea urchins, yeast and Salmonella typhimurium. Teratog. Carcinog. Mutag. 3, 377-393.

Pagano G, Cipollaro M, Corsale G, Della Morte RD, Esposito, A, Giordano GG, Micallo G, Quinto I and Staiano N, 1988. Comparitive toxicity of diphenyl, diphenyl ether, and some of their hydroxy derivatives. Med. Biol. Environ. 16, 291-297.

Rapson WH, Nazar MA and Butzky VV, 1980. Mutagenicity produced by aqueous chlorination of organic compounds. Bull. Environ. Contam. Toxicol. 24, 590-596.

San Sebastian JR, 1989b. In vitro chromosome aberration analysis in Chinese hamster ovary (CHO) cells. Diphenyl oxide, 1-methoxy-2-propane, p-nitrophenol, dimethylformamide, o-benzyl-p-chlorophenol, triethanolamine. EPA Doc. 86-890000343. June 8, 1989. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Sasaki YF, Imanishi H, Ohta T and Yasuhiko S, 1989. Modifying effects of components of plant essence on the induction of sister-chromatid exchanges in cultured Chinese hamster ovary cells. Mutat. Res. 226, 103-110.

SCF, 1999a. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Venkov P, Topashka-Ancheva M, Georgieva M, Alexieva V and Karanov E, 2000. Genotoxic effect of substituted phenoxyacetic acids. Arch. Toxicol. 74(9), 560-566.

Westinghouse Electric Corporation, 1984. Submission to EPA. Unpublished report to the Flavor and Extract Manufacturers Association. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

Wild D, King MT, Gocke E and Eckhard K, 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food Chem. Toxicol. 21(6), 707-719.

Yoo YS, 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka City Med. J. 34(3-4), 267-288.

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
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
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
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
WHO	World Health Organisation