EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 23, Revision 3 (FGE.23Rev3): Aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30

EFSA Publication

Link to article, DOI:
[10.2903/j.efsa.2011.2398](https://doi.org/10.2903/j.efsa.2011.2398)

Publication date:
2011

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

[Link back to DTU Orbit](#)

Citation (APA):
EFSA Publication (2011). *EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 23, Revision 3 (FGE.23Rev3): Aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30*. European Food Safety Authority. EFSA Journal No. 2398 <https://doi.org/10.2903/j.efsa.2011.2398>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 23, Revision 3 (FGE.23Rev3):

Aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30¹

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 20 flavouring substances in the Flavouring Group Evaluation 23, Revision 3, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that all 20 substances [FL-no: 02.247, 02.248, 03.008, 03.011, 03.012, 03.015, 03.016, 03.020, 03.022, 03.024, 04.059, 04.067, 04.068, 04.069, 04.075, 04.079, 04.084, 08.127, 09.687 and 13.200] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all 20 candidate substances.

© European Food Safety Authority, 2011

1 On request from the Commission, Question No EFSA-Q-2011-00862, adopted on 29 September 2011.

2 Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Leon Brimer, Laurence Castle, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Fidel Toldra, Detlef Wölfl.

Correspondence: cef-unit@efsa.europa.eu

3 The 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, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific opinion.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 23, Revision 3 (FGE.23Rev3):

Aliphatic, alicyclic and aromatic ethers including anisole derivatives from chemical groups 15, 16, 22, 26 and 30. EFSA Journal 2011; 9(10):2398. [72 pp.]. doi:10.2903/j.efsa.2011.2398. 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 asked to evaluate one flavouring substance in the Flavouring Group Evaluation 23, Revision 3 (FGE.23Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The flavouring substance belongs to chemical group 16, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation therefore deals with 20 candidate substances, which are aliphatic, alicyclic and aromatic ethers including anisole derivatives. Four of the candidate substances are aliphatic ethers, two are alicyclic ethers, three are alicyclic hydrocarbons with an ether side chain, two are ethers containing a benzene moiety, eight are phenol ethers and one is a naphthol ether.

Six of the 20 candidate substances possess one or more chiral centres and three can exist as geometrical isomers. For these six substances the stereoisomeric composition has been specified. Three candidate substances can exist as geometrical isomers, the isomers have been specified.

Two of the flavouring substances are classified into structural class I, seven are classified into structural class II and 11 are classified into structural class III according to the decision tree approach presented by Cramer et al., 1978.

Ten of the substances in the present group have been reported to occur naturally in a wide range of food items.

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

In the absence of more precise 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. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the 20 flavouring substances in this group have intakes in Europe from 0.011 to 49 micrograms/*capita*/day, which are below the threshold of concern value for structural class I of 1800 micrograms/person/day, for structural class II of 540 micrograms/person/day and for structural class III of 90 micrograms/person/day.

The genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

According to the available data on supporting substances, it is expected that all 20 candidate substances in this group would be metabolised to innocuous products at the reported levels of intake as flavouring substances.

It was noted that no repeated dose toxicity studies have been provided for any of the candidate substances and only a few studies were available on supporting substances. However, these toxicological data were consistent with the conclusions in the present Flavouring Group Evaluation using the Procedure.

It was concluded that on the basis of the default MSDI approach the 20 candidate substances would not give rise to safety concerns at estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they were 3200 micrograms/person/day for the two flavouring substances belonging to structural class I and for five of the seven flavouring substances belonging to structural class II. The mTAMDI for the remaining two flavouring substances from class II are 3500 and 14000 micrograms/person/day. These intakes are above the threshold of concern for structural class I of 1800 micrograms/person/day and for structural class II of 540 micrograms/person/day. For 10 of the 11 candidate substances belonging to structural class III the mTAMDI are 860, 2500, 3200 or 3900 microgram/person/day, which are above the threshold of concern of 90 micrograms/person/day. For one substance from structural class III the mTAMDI of 58 micrograms/person/day is below the threshold. This substance is also expected to be metabolised to innocuous products.

Thus, for 19 of the 20 flavouring substances considered in this Opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substances have been assigned. Therefore, for these 19 substances, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the 20 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including purity criteria and identity for the materials of commerce have been provided for all 20 flavouring substances.

For all the 20 substances [FL-no: 02.247, 02.248, 03.008, 03.011, 03.012, 03.015, 03.016, 03.020, 03.022, 03.024, 04.059, 04.067, 04.068, 04.069, 04.075, 04.079, 04.084, 08.127, 09.687 and 13.200] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

KEYWORDS

Flavourings, food safety, aliphatic, alicyclic, aromatic, ethers, FGE.23.

TABLE OF CONTENTS

Abstract	1
Summary	2
Keywords	3
Table of contents	4
Background	5
History of the Evaluation	5
Terms of Reference	6
Assessment	6
1. Presentation of the Substances in Flavouring Group Evaluation 23, Revision 3	6
1.1. Description	6
1.2. Stereoisomers	7
1.3. Natural Occurrence in Food	7
2. Specifications	8
3. Intake Data	8
3.1. Estimated Daily <i>per Capita</i> Intake (MSDI Approach)	8
3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)	9
4. Absorption, Distribution, Metabolism and Elimination	10
5. Application of the Procedure for the Safety Evaluation of Flavouring Substances	13
6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach	14
7. Considerations of Combined Intakes from Use as Flavouring Substances	15
8. Toxicity	16
8.1. Acute Toxicity	16
8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies	16
8.3. Developmental / Reproductive Toxicity Studies	16
8.4. Genotoxicity Studies	17
9. Conclusions	17
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 23, Revision 3	20
Table 2a: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)	23
Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters	26
Table 3: Supporting Substances Summary	27
Annex I: Procedure for the Safety Evaluation	30
Annex II: Use Levels / mTAMDI	32
Annex III: Metabolism	36
Annex IV: Toxicity	51
References	61
Abbreviations	71

BACKGROUND

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

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The Flavouring Group Evaluation (FGE) is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

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

HISTORY OF THE EVALUATION

The Flavouring Group Evaluation 23, FGE.23 dealt with 14 aliphatic, alicyclic and aromatic ethers including anisole derivatives.

The first Revision of FGE.23, FGE.23Rev1, included the assessment of four additional candidate substances [FL-no: 02.247, 02.248, 03.022 and 08.127]. No toxicity or metabolism data were available, except for one new substance [FL-no: 02.248], for which two *in vitro* genotoxicity studies have been submitted. Additional information on stereoisomeric composition on two substances [FL-no: 03.008 and 03.020] has become available since the first FGE was published.

The second Revision of FGE.23, FGE.23Rev2, included the assessment of one additional candidate substance [FL-no: 03.024]. No toxicity and/or metabolism data were provided by Industry for this substance. A search in open literature for this substance did not provide any further data on toxicity or metabolism. Since the publication of FGE.23Rev1 information on stereoisomeric composition and a identity test has been provided by EFFA on the following four substances: [FL-no: 02.247, 02.248, 03.022 and 08.127].

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.23	29 November 2006	http://www.efsa.europa.eu/en/science/afc/afc_opinions/ej417_fge23.html	14
FGE.23Rev1	27 September 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902124677.htm	18
FGE.23Rev2	29 September 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1848.htm	19

The present revision of FGE.23, FGE.23Rev3, includes the assessment of one additional candidate substance 5-isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran [FL-no: 13.200]. No new metabolism data were provided by Industry for this substance. However, for the supporting substance [FL-no: 13.037] *in vitro* genotoxicity study was provided. A search in open literature for [FL-no: 13.200] did not provide any further data on toxicity or metabolism.

Since the publication of FGE.23Rev2 information on stereoisomeric composition and use levels has been provided by EFSA on the following two substances: [FL-no: 02.248 and 03.022] (EFSA, 2011a; Flavour Industry, 2011b).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letter of the 12th April 2010, the Commission requested EFSA to carry out an evaluation of the flavouring substance 5-isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran [FL-no: 13.200], also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 23, Revision 3

1.1. Description

The present Flavouring Group Evaluation 23, Revision 3 (FGE.23Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (The Procedure – shown in schematic form in Annex I of this FGE), deals with 20 aliphatic, alicyclic or aromatic ethers. These 20 flavouring substances (candidate substances) belong to the chemical groups 15, 16, 22, 26 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 20 candidate substances under consideration in the present evaluation are listed in Table 1, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structures and specifications. Four of the candidate substances are aliphatic ethers [FL-no: 03.015, 03.016, 03.022 and 03.024], two are alicyclic ethers [FL-no: 03.008 and 13.200], three are alicyclic hydrocarbons with ether side chain [FL-no: 02.247, 02.248 and 03.020] of which [FL-no: 02.248] also has an acetal moiety, two are ethers containing a benzene moiety [FL-no: 03.011 and 03.012], eight are phenol ethers [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687] and one is a naphthol ether [FL-no: 04.075].

The outcome of the safety evaluations are summarised in Table 2a.

The hydrolysis products of the candidate esters and the acetal are listed in Table 2b.

The 20 candidate substances are structurally related to 28 flavouring substances (supporting substances) evaluated at the 51st JECFA meeting (JECFA, 2000a) in the group of “Aliphatic acyclic and alicyclic terpenoid tertiary alcohols and structurally related substances”, evaluated at the 59th JECFA meeting (JECFA, 2002c) in the group of “Phenethyl alcohol, aldehyde, acid and related acetals and esters” and evaluated at the 61st JECFA meeting (JECFA, 2004a) in the group of “Aliphatic and aromatic ethers”. These substances, with the respective structural formulas, FEMA, CoE and CAS register numbers, evaluation status by the Scientific Committee on Food (SCF), the JECFA and the CoE, and the European Maximised Survey-derived Daily Intake (MSDI) values, are listed in Table 3.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

Two flavouring substances possess one chiral centre [FL-no: 03.020 and 08.127], three possess three chiral centres [FL-no: 02.247, 03.008 and 13.200] and one possesses five chiral centres [FL-no: 02.248]. The stereoisomeric composition has been specified for all these substances (see Table 1).

Due to the presence and the position of double bonds three of the substances [FL-no: 03.015, 03.022 and 03.024] can exist as geometrical isomers. The chemical Register name and the CASrn provided specify the configuration of the double bond for [FL-no: 03.015 and 03.024]. For [FL-no: 03.022] Industry has informed that it occurs as a mixture of E- & Z-isomers with at least 40 % of each (EFFA, 2011a) (see Table 1).

1.3. Natural Occurrence in Food

Ten candidate substances in the present group have been reported to occur in spices (ginger, savory, vanilla, thyme, clary sage, marjoram), dried bonito, tea, juice (grapefruit, lemon), lychee fruit, starfruit, heated blackberry, heated beans, cape gooseberry, mushroom, smoked oily fish, brandy, rum and wine (TNO, 2000). Quantitative data on the natural occurrence in foods have been reported for three of these substances in the present Flavouring Group Evaluation.

These reports include:

- Ethyl geranyl ether [FL-no: 03.015]: 0.0001 mg/kg in grapefruit juice, up to 0.2 mg/kg in lychee
- Carvacryl methyl ether [FL-no: 04.059]: 800 mg/kg in ginger, up to 5000 mg/kg in savory, up to 14400 mg/kg in thyme
- 1,2,3-Trimethoxybenzene [FL-no: 04.084]: 3.8 mg/kg in dried bonito, 20 mg/kg in tea.

According to TNO (TNO, 2000; TNO, 2011) 10 of the substances have not been reported to occur naturally in any food items. These 10 substances are l-menthoxyethanol [FL-no: 02.247], vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248], 2-acetoxy-1,8-cineole [FL-no: 03.008], alpha-terpinyl methyl ether [FL-no: 03.020], 1-methoxy-1-decene [FL-no: 03.022], digeranylether [FL-no: 03.024], 1-ethoxy-2-methoxybenzene [FL-no: 04.067], 2-(4-methoxyphenoxy)propionic acid [FL-no:

08.127], 2-phenoxyethyl butyrate [FL-no: 09.687] and 5-isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran [FL-no: 13.200].

2. Specifications

Purity criteria for the 20 substances have been provided by the flavouring industry (EFFA, 2003k; EFFA, 2004af; EFFA, 2004j; Flavour Industry, 2006a; Flavour Industry, 2009g; Flavour Industry, 2010i) (Table 1).

Judged against the requirements in Annex II of Commission Regulation EC No 1565/2000 (EC, 2000), the information is adequate for all 20 candidate substances (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during

the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population⁴ (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present Flavouring Group Evaluation 23, Revision 3 (FGE.23Rev3) the total annual volume of production of the 20 candidate substances for use as flavouring substances in Europe has been reported to be approximately 750 kg (EFFA, 2003l; EFFA, 2004af; EFFA, 2004k; Flavour Industry, 2006a; Flavour Industry, 2009g; Flavour Industry, 2010i) and for 28 supporting substances approximately 34000 kg (JECFA, 2000a; JECFA, 2002c; JECFA, 2004a).

On the basis of the annual volumes of production reported for the 20 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a). Approximately 96 % of the total annual volume of production for the candidate substances is accounted for by seven flavourings: 1-methoxyethanol [FL-no: 02.247], benzyl methyl ether [FL-no: 03.011], alpha-terpinyl methyl ether [FL-no: 03.020], 1-methoxy-1-decene [FL-no: 03.022], digeranyl ether [FL-no: 03.024], carvacryl methyl ether [FL-no: 04.059] and 5-isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran [FL-no: 13.200]. The estimated daily *per capita* intakes of these candidate substances from use as a flavouring substance are 15, 1.9, 4.1, 6.1, 49, 1.2 and 12 microgram, respectively. For each of the remaining 13 substances the estimated daily *per capita* intake is less than 0.7 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the 20 candidate substances, information on food categories and normal and maximum use levels^{5,6,7} were submitted, by the Flavour Industry (EFFA, 2003k; EFFA, 2004af; EFFA, 2004j; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2008a; Flavour Industry, 2009g; Flavour Industry, 2010i; Flavour Industry, 2011b).

The 20 candidate substances, for which normal and maximum use levels were submitted by Industry, are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

⁴ EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

⁵ "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

⁶ The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

⁷ The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except [FL-no: 02.248 and 03.024]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 03.024]
03.0	Edible ices, including sherbet and sorbet	All except [FL-no: 02.248]
04.1	Processed fruits	All except [FL-no: 02.248 and 03.024]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 02.248, 03.022 and 13.200]
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 03.022 and 03.024]
07.0	Bakery wares	All except [FL-no: 03.024]
08.0	Meat and meat products, including poultry and game	All except [FL-no: 02.248, 03.024 and 13.200]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 03.024 and 13.200]
10.0	Eggs and egg products	Only [FL-no: 13.200]
11.0	Sweeteners, including honey	Only [FL-no: 13.200]
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no: 03.024]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 02.248, 03.022, 03.024 and 13.200]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All
15.0	Ready-to-eat savouries	All except [FL-no: 02.248, 03.008, 03.024 and 13.200]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no: 13.200]

According to the Flavour Industry the normal use levels for the 20 candidate substances, are in the range of 0.0015 to 70 mg/kg food and the maximum use levels are in the range of 0.0125 to 100 mg/kg (EFFA, 2003k; EFFA, 2004af; EFFA, 2004j; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2008a; Flavour Industry, 2009g; Flavour Industry, 2010i; Flavour Industry, 2011b) (see Table II.1.2, Appendix II).

The mTAMDI values for the 20 candidate substances from structural class I, II and III (see Section 5) are in the range of 58 to 14000 micrograms/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. Absorption, Distribution, Metabolism and Elimination

The candidate substances are examples of aliphatic, alicyclic or aromatic ethers. On the basis of their structure they can be divided into seven subgroups:

1) aliphatic ethers [FL-no: 03.015, 03.016 and 03.022],

- 2) alicyclic ethers [FL-no: 03.008 and 13.200],
- 3) alicyclic hydrocarbons with an ether side chain [FL-no: 02.247, 02.248 and 03.020] of which [FL-no: 02.248] also has an acetal moiety,
- 4) benzyl ethers [FL-no: 03.011 and 03.012],
- 5) phenol ethers [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687],
- 6) naphthol ethers [FL-no: 04.075] and
- 7) long chain aliphatic ethers [FL-no: 03.024] (see Table 4.1).

No data on absorption, distribution, metabolism or elimination are reported for 19 of the 20 candidate substances.

According to the available data on supporting substances, the simple aliphatic ethers in subgroup 1 [FL-no: 03.015, 03.016 and 03.022], the cyclic ethers in subgroup 2 [FL-no: 03.008 and 13.200], the cyclic hydrocarbons with ether side chain in subgroup 3 [FL-no: 02.247, 02.248 and 03.020] of which [FL-no: 02.248] also has an acetal moiety, the benzyl ethers in subgroup 4 [FL-no: 03.011 and 03.012] and the phenolic ethers in subgroup 5 [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687] and the long chain aliphatic ether in subgroup 7) [FL-no: 03.024] are all expected to be rapidly absorbed from the gastrointestinal tract and excreted in the exhaled air as CO₂ and as polar metabolites in the urine.

After absorption, the supporting substance, beta-naphthyl methyl ether [FL-no: 04.033], representative for the naphthol ether in subgroup 6 [FL-no: 04.075] is hydroxylated and excreted as a glucuronide.

Concerning their biotransformation, it can be expected that the straight-chain aliphatic ethers included in subgroup 1 may undergo O-dealkylation *in vivo*, catalysed by cytochrome P450 to yield the corresponding alcohol and aldehyde that subsequently undergo complete oxidation in the fatty acid pathway and tricarboxylic acid cycle. The demethylated product of 1-methoxy-1-decene [FL-no: 03.022] is an enol, which will rearrange to the aldehyde, which subsequently can be oxidised to the carboxylic acid.

The candidate substances, 2-acetoxy-1,8-cineole [FL-no: 03.008] and 5-isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran [FL-no: 13.200], within subgroup 2 (alicyclic ether), on the basis of information on representative supporting substances, may be anticipated to undergo ring-hydroxylation by P450, conjugation with glucuronic acid followed by excretion in the urine.

The available data on alpha-terpineol [FL-no: 02.014] and terpenoid tertiary alcohols, taken as supporting substances, suggest that the substance in subgroup 3 [FL-no: 03.020] would be metabolised by P450 isoenzymes to yield polar hydroxylated metabolites, which are conjugated to form glucuronic acid conjugates and excreted or are further oxidised and excreted. Cleavage of the ether is a minor metabolic pathway (JECFA, 1999a). The acetal moiety in vanillin 3-(1-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] is shown to be hydrolysed, resulting in the formation of the corresponding ether and vanillin. It is expected that the alcohol group in this ether subsequently are oxidised and that the carboxylic acid(s) are excreted as a conjugate or excreted as the acid itself. Similarly, 1-menthoxy ethanol [FL-no: 02.247] is anticipated to be oxidised to the corresponding carboxylic acid and excreted.

The benzyl ethers found in subgroup 4 [FL-no: 03.011 and 03.012] are expected to be metabolised in a similar way to mono-alkyl derivatives of benzene. It is generally accepted that mono-alkyl derivatives of benzene are metabolised by undergoing biotransformation of the side chain to produce alcohols and carboxylic acids which are eliminated in the urine as conjugates of glucuronic acid or glycine (Williams, 1959a).

The candidate aromatic ethers in subgroup 5 [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687] are expected to be metabolised by ring-hydroxylation (mainly in the *para* position, cleavage of the methyl ether (O-demethylation) and/or oxidation of the ring substituents depending on the position of substituents. These products would then be expected to be conjugated primarily with glucuronic acid and to a lesser extent sulphate or glycine and excreted in the urine. 2-(4-Methoxyphenoxy)propionic acid [FL-no: 08.127] is expected to be excreted after conjugation or alternatively excreted as the unconjugated acid.

Table 4.1. Candidate Substances Divided into Subgroups of Related Chemical Structures

FL-no	EU Register name	Structural formula
1 Aliphatic Ethers		
03.015	Ethyl geranyl ether
03.016	Hexyl methyl ether
03.022	1-Methoxy-1-decene
2 Alicyclic Ethers		
03.008	2-Acetoxy-1,8-cineole
13.200	5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran
3 Alicyclic Hydrocarbons with Ether Side Chain		
02.247	l-Menthoxylethanol
02.248	Vanillin 3-(l-menthoxy)propane-1,2-diol acetal
03.020	alpha-Terpinyl methyl ether
4 Benzyl Ethers		
03.011	Benzyl methyl ether
03.012	Benzyl octyl ether
5 Phenol Ethers		
04.059	Carvacryl methyl ether
04.067	1-Ethoxy-2-methoxybenzene
04.068	1-Ethoxy-4-methoxybenzene
04.069	1-Ethyl-4-methoxybenzene
04.079	Methyl 4-methoxybenzyl ether
04.084	1,2,3-Trimethoxybenzene
08.127	2-(4-Methoxyphenoxy)propionic acid
09.687	2-Phenoxyethyl butyrate
6 Naphthol Ethers		
04.075	1-Methoxynaphthalene
7 Long Chain Aliphatic Ether		

Table 4.1. Candidate Substances Divided into Subgroups of Related Chemical Structures

FL-no	EU Register name	Structural formula
03.024	Digeranyl ether

Metabolism data are available on the supporting substance 2-methoxynaphthalene [FL-no: 04.074] from subgroup 6, which is shown to be excreted as a glucuronide (Williams, 1959a).

No data are available on the absorption, distribution and excretion of the substance in subgroup 7 or any supporting substances but as with the other substances in this FGE, digeranyl ether would be expected to be rapidly absorbed from the gastrointestinal tract and excreted as polar metabolites in the urine and in the exhaled air as CO₂.

Whilst no metabolism data have been found for the candidate substance in subgroup 7, digeranyl ether [FL-no: 03.024], data are available on substances that are supporting for the metabolism of longer chain ethers. Dealkylation of ethers becomes less likely as the chain length increases and ω -oxidation is more likely to occur (Tsuji et al., 1978). For geraniol, next to other pathways of metabolism related to the presence of a free hydroxyl-group, ω -oxidation has also been described and this would result in metabolism to innocuous products. As digeranyl ether contains no free hydroxyl group like geraniol, the other pathways for metabolism of geraniol are not available for digeranyl ether and therefore ω -oxidation is more likely to occur. It would be expected that following ω -oxidation, the metabolites would be conjugated with glucuronide and excreted in the urine.

It can be anticipated that all 20 candidate substances [FL-no: 02.247, 02.248, 03.008, 03.011, 03.012, 03.015, 03.016, 03.020, 03.022, 03.024, 04.059, 04.067, 04.068, 04.069, 04.075, 04.079, 04.084, 08.127, 09.687 and 13.200] are metabolised to innocuous products. Although saturation of some metabolic pathways have been described, it occurs at high doses, unlikely to be reached by the candidate substances when used as flavouring substances at the present level of intake.

A more detailed discussion of the metabolism of the candidate substances in this evaluation is provided in Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the 20 candidate substances from chemical groups 15, 16, 22, 26 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations are summarised in Table 2a.

Step 1

Using the decision tree approach presented by Cramer *et al.*, two of the candidate substances [FL-no: 04.059 and 04.084] were classified into structural class I, seven [FL-no: 03.008, 03.011, 03.012, 03.015, 03.016, 03.024 and 04.079] into structural class II and 11 substances [FL-no: 02.247, 02.248, 03.020, 03.022, 04.067, 04.068, 04.069, 04.075, 08.127, 09.687 and 13.200] into structural class III (Cramer et al., 1978).

Step 2

On the basis of the metabolism information available all 20 candidate substances [FL-no: 02.247, 02.248, 03.008, 03.011, 03.012, 03.015, 03.016, 03.020, 03.022, 03.024, 04.059, 04.067, 04.068, 04.069, 04.075, 04.079, 04.084, 08.127, 09.687 and 13.200] can be predicted to be metabolised to innocuous products and therefore they will proceed along the A-side of the Procedure scheme.

Step A3

Two candidate substances [FL-no: 04.059 and 04.084] have been assigned to structural class I and have estimated European daily *per capita* intakes (MSDI) of 0.012 and 1.2 micrograms. The seven candidate substances [FL-no: 03.008, 03.011, 03.012, 03.015, 03.016, 03.024 and 04.079], which have been assigned to structural class II have estimated European daily *per capita* intake ranging from 0.012 to 49 micrograms and the 11 candidate substances [FL-no: 02.247, 02.248, 03.020, 03.022, 04.067, 04.068, 04.069, 04.075, 08.127, 09.687 and 13.200], which have been assigned to structural class III, have estimated European daily *per capita* intake ranging from 0.011 to 15 micrograms (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 micrograms/person/day for structural class I, II and III, respectively.

Based on results of the safety evaluation sequence of the Procedure, these 20 candidate substances, proceeding via the A-side of the Procedure scheme, do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for the 20 candidate substances in structural class I, II and III based on the mTAMDI range from 58 to 14000 micrograms/person/day. For 19 of these substances the mTAMDI is above the threshold of concern of 1800 micrograms/person/day for structural class I, of 540 micrograms/person/day for structural class II and of 90 micrograms/person/day for structural class III. The estimated intake for [FL-no: 03.022] in structural class III based on the mTAMDI is 58 micrograms/person/day, which is below the threshold of concern. This substance is also expected to be metabolised to innocuous products.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

For 19 candidate substances, for which the mTAMDI is above the threshold of concern further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, see Table 6.1.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/capita/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
04.059	Carvacryl methyl ether	1.2	3200	Class I	1800
04.084	1,2,3-Trimethoxybenzene	0.012	3200	Class I	1800
03.008	2-Acetoxy-1,8-cineole	0.037	3500	Class II	540
03.011	Benzyl methyl ether	1.9	3200	Class II	540
03.012	Benzyl octyl ether	0.24	3200	Class II	540
03.015	Ethyl geranyl ether	0.012	3200	Class II	540
03.016	Hexyl methyl ether	0.012	3200	Class II	540
03.024	Digeranyl ether	49	14000	Class II	540
04.079	Methyl-4-methoxybenzyl ether	0.61	3200	Class II	540
02.247	l-Menthoxyethanol	15	3900	Class III	90

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI ($\mu\text{g}/\text{capita}/\text{day}$)	mTAMDI ($\mu\text{g}/\text{person}/\text{day}$)	Structural class	Threshold of concern ($\mu\text{g}/\text{person}/\text{day}$)
02.248	Vanillin 3-(1-menthoxy)propane-1,2-diol acetal	0.61	2500	Class III	90
03.020	alpha-Terpinyl methyl ether	4.1	3200	Class III	90
03.022	1-Methoxy-1-decene	6.1	58	Class III	90
04.067	1-Ethoxy-2-methoxybenzene	0.12	3200	Class III	90
04.068	1-Ethoxy-4-methoxybenzene	0.67	3200	Class III	90
04.069	1-Ethyl-4-methoxybenzene	0.073	3200	Class III	90
04.075	1-Methoxynaphthalene	0.061	3200	Class III	90
08.127	2-(4-Methoxyphenoxy)propionic acid	0.011	3200	Class III	90
09.687	2-Phenoxyethyl butyrate	0.085	3900	Class III	90
13.200	5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran	12	860	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003l; EFFA, 2004af; EFFA, 2004k; Flavour Industry, 2006a; Flavour Industry, 2009g; Flavour Industry, 2010i), the combined estimated daily *per capita* intakes as flavourings of the two candidate substances assigned to structural class I, of the seven candidate substances belonging to structural class II and of the 11 candidate substances belonging to structural class III are 1.2, 52 and 39 micrograms, respectively. These values do not exceed the threshold of concern for substances belonging to structural class I of 1800 micrograms/person/day, structural class II of 540 microgram/person/day and structural class III of 90 micrograms/person/day.

The 20 candidate substances are structurally related to 28 supporting substances evaluated by the JEFCA at its 51st, 59th and 61st meeting (JECFA, 2000a; JECFA, 2002c; JECFA, 2004a). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for the 28 supporting substances.

The total combined intake of the two candidate substances and 11 supporting substances from structural class I, is approximately 2800 micrograms/*capita*/day, which exceeds the threshold of concern for a compound belonging to structural class I of 1800 micrograms/*capita*/day. However, the major contribution to the total combined intake of flavouring substances assigned to structural class I (99 %) is provided by the two supporting substances, namely alpha-terpineol [FL-no: 02.014] (2600 micrograms/*capita*/day) and terpineol acetate [FL-no: 09.830] (220 micrograms/*capita*/day). Terpineol acetate is anticipated to be hydrolysed to alpha-terpineol. These supporting substances were evaluated at the 51st JECFA meeting, where it was noted that although the combined intake exceeds the threshold for structural class I the substances are expected to be efficiently metabolised and would not saturate the metabolic pathways. The Panel agreed with this view and concluded that the combined intake of about 1.2 micrograms/*capita*/day for the candidate substances in structural class I is negligible compared to the combined intake of 2800 micrograms/*capita*/day of the supporting substances.

The total combined intake of the seven candidate substances and 10 supporting substances from structural class II, is approximately 1300 micrograms/*capita*/day, which exceeds the threshold of concern for a compound belonging to structural class II of 540 micrograms/person/day. More than 95 % of the combined daily *per capita* intake of 1250 micrograms is provided by the supporting substance 1,8-cineole [FL-no: 03.001]. The supporting substances in structural class II were evaluated at the 61st JECFA meeting, where it was noted that although the combined intake exceeds the threshold, the substances are expected to be efficiently metabolised and would not saturate the metabolic pathways. The Panel agreed with this view and concluded that the combined intake of about 52 micrograms/*capita*/day for the candidate substances in structural class II is negligible compared to the combined intake of 1250 micrograms/*capita*/day of the supporting substances.

The total combined intake of the 11 candidate substances and seven supporting substances from structural class III for which production volumes in Europe were reported, is approximately 140 micrograms/*capita*/day, which exceed the threshold of concern for a substance belonging to structural class III of 90 micrograms/*capita*/day.

The supporting substances were evaluated by the JECFA at the 59th and 61st meetings, where it was noted that although the combined intake exceeds the threshold for the structural class, the substances are expected to be efficiently metabolised and would not saturate the metabolic pathways. The Panel agreed with this view and concluded that the combined intake of about 39 micrograms/*capita*/day for the candidate substances in structural class III is minor compared to the combined intake of about 100 micrograms/*capita*/day of the supporting substances.

Therefore, it can be concluded that the total combined intakes of the 20 candidate substances and 28 supporting substances, including 1,8-cineole [FL-no: 03.001], alpha-terpineol [Fl-no: 02.014] and terpineol acetate [FL-no: 09.830], do not pose a safety concern.

8. Toxicity

8.1. Acute Toxicity

Data are available for one of the candidate substances, ethyl-geranyl ether [FL-no: 03.015] with an oral LD₅₀ value of more than 5000 mg/kg body weight (bw).

Twenty of the 28 supporting substances were tested for acute toxicity in mice and/or rats. The oral LD₅₀ values in mice and rats for the supporting substances range from 1000 mg/kg to 8000 mg/kg bw.

Acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data on subacute and subchronic toxicity are not available for any of the candidate substances but for 10 of the 28 supporting substances of the present flavouring group.

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data on developmental toxicity and reproductive toxicity data are not available for any of the candidate substances but for two of the 28 supporting substances of the present flavouring group, 1,8-cineole [FL-no: 03.001] and phenoxyacetic acid [FL-no: 08.049].

The data available on developmental / reproductive toxicity do not preclude the evaluation of the candidate substances through the Procedure.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

There are only five genotoxicity studies carried out on the candidate substances, 1,2,3-trimethoxybenzene [FL-no: 04.084], vanillin 3-(1-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] and 2,6-diethyl-5-isopropyl-2-methyltetrahydropyran [FL-no: 13.200]. One reverse mutation assay on [FL-no: 13.200] was considered valid and gave negative results both with and without metabolic activation (Thompson, 2008). The four remaining 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 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., 1987a). This study was only positive without S9 activation and at levels of 1,8-cineole of 200 and 500 microgram/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., 1987a) is considered not to be of relevance for the overall evaluation. It is therefore concluded that 1,8-cineole is not genotoxic.

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

Genotoxicity data are summarised in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The 20 candidate substances are aliphatic, alicyclic and aromatic ethers including anisole derivatives and belong to EU chemical groups 15, 16, 22, 26 and 30. Four of the candidate substances are aliphatic ethers, two are alicyclic ethers, three are alicyclic hydrocarbons with an ether side chain, two are ethers containing a benzene moiety, eight are phenol ethers and one is a naphthol ether.

Six of the 20 candidate substances possess one or more chiral centres. For these six substances the stereoisomeric composition has been specified. Three candidate substances can exist as geometrical isomers, the isomers have been specified.

Two of the flavouring substances are classified into structural class I, seven are classified into structural class II and 11 are classified into structural class III.

Ten of the substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the 20 flavouring substances in this group have intakes in Europe from 0.011 to 49 micrograms/*capita*/day, which are below the threshold of concern value for

structural class I of 1800 micrograms/person/day, for structural class II of 540 micrograms/person/day and for structural class III of 90 micrograms/person/day.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the two candidate substances belonging to structural class I, of the seven candidate substances belonging to structural class II and of the 11 candidate substances belonging to structural class III, would result in combined intakes of approximately 1.2, 52 and 39 micrograms/*capita*/day, respectively. These values are lower than the thresholds of concern for structural class I, II or III substances. The estimated total combined intakes of the candidate and supporting substances (in Europe) are approximately 2800, 1300 and 140 micrograms/*capita*/day for structural class I, II and III substances, respectively.

The combined daily *per capita* intake of 2800 microgram exceeds the threshold of concern of 1800 microgram/person/day for structural class I substances. The supporting substances were evaluated at the 51st JECFA meeting, where it was noted that although the combined intake exceeds the threshold for structural class I the substances are expected to be efficiently metabolised and would not saturate the metabolic pathways. The Panel agreed with this view and concluded that the combined intake of about 1.2 micrograms/*capita*/day for the candidate substances in structural class I is negligible compared to the combined intake of 2800 micrograms/*capita*/day of the supporting substances.

Likewise the total combined intake of the seven candidate substances and 10 supporting substances from structural class II is approximately 1300 micrograms/*capita*/day, which exceeds the threshold of concern for a compound belonging to structural class II of 540 micrograms/person/day. The supporting substances in structural class II were evaluated at the 61st JECFA meeting, where it was noted that although the combined intake exceeds the threshold, the substances are expected to be efficiently metabolised and would not saturate the metabolic pathways. The Panel agreed with this view and concluded that the combined intake of about 52 micrograms/*capita*/day for the candidate substances in structural class II is negligible compared to the combined intake of 1250 micrograms/*capita*/day of the supporting substances.

The total combined intake of candidate and supporting substances of structural class III is 140 micrograms/*capita*/day, which is above the threshold of concern for structural class III of 90 micrograms/*capita*/day. The supporting substances were evaluated by the JECFA at the 59th and 61st meetings, where it was noted that although the combined intake exceeds the threshold for the structural class, the substances are expected to be efficiently metabolised and would not saturate the metabolic pathways. The Panel agreed with this view and concluded that the combined intake of about 39 micrograms/*capita*/day for the candidate substances in structural class III is minor compared to the combined intake of 100 micrograms/*capita*/day of the supporting substances.

The genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

According to the available data on supporting substances, it is expected that all 20 candidate substances in this group [FL-no: 02.247, 02.248, 03.008, 03.011, 03.012, 03.015, 03.016, 03.020, 03.022, 03.024, 04.059, 04.067, 04.068, 04.069, 04.075, 04.079, 04.084, 08.127, 09.687 and 13.200] would be metabolised to innocuous products at the reported levels of intake as flavouring substances.

It was noted that no repeated dose toxicity studies have been provided for any of the candidate substances and only a few studies were available on supporting substances. However, these toxicological data were consistent with the conclusions in the present Flavouring Group Evaluation using the Procedure.

It was concluded that on the basis of the default MSDI approach the 20 candidate substances would not give rise to safety concerns at estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they were 3200 micrograms/person/day for the two flavouring substances belonging to structural class I and for five of the seven flavouring substances belonging to structural class II. The mTAMDI for the two remaining flavouring substances from class II are 3500 and 14000 micrograms/person/day. These intakes are above the threshold of concern for structural class I of 1800 micrograms/person/day and for structural class II of 540 micrograms/person/day. For 10 candidate substances belonging to structural class III the mTAMDI are 860, 2500, 3200 or 3900 microgram/person/day, which are above the threshold of concern of 90 micrograms/person/day. For one substance from structural class III the mTAMDI of 58 micrograms/person/day is below the threshold. This substance is also expected to be metabolised to innocuous products.

Thus, for 19 of the 20 flavouring substances considered in this Opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substances have been assigned. Therefore, for these 19 substances, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the 20 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including purity criteria and identity for the materials of commerce have been provided for all 20 flavouring substances.

For all the 20 candidate substances [FL-no: 02.247, 02.248, 03.008, 03.011, 03.012, 03.015, 03.016, 03.020, 03.022, 03.024, 04.059, 04.067, 04.068, 04.069, 04.075, 04.079, 04.084, 08.127, 09.687 and 13.200] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 23, REVISION 3

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 23, Revision 3

FL-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)	Specification comments
02.247	l-Menthoxylethanol		4154 38618-23-4	Liquid C ₁₂ H ₂₄ O ₂ 200.32	Practically insoluble or insoluble Freely soluble	100 IR NMR MS 99 %	1.457-1.467 0.930-0.950	Register name to be changed to L-1-Menthoxylethanol.
02.248	Vanillin 3-(l-menthoxy)propane-1,2-diol acetal		3904 180964-47-0	Solid C ₂₁ H ₃₂ O ₅ 364.49	Very slightly soluble Freely Soluble	78-80 IR NMR 97 %	n.a. n.a.	Mixture of four stereoisomers with equal ratios of the isomers (EFFA, 2010a).
03.008	2-Acetoxy-1,8-cineole		57709-95-2	Solid C ₁₂ H ₂₀ O ₃ 212.29	Practically insoluble or insoluble Freely soluble	299 89 MS 95 %	n.a. n.a.	Register name to be changed to (1R, 4S, 6S)-2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, 6-acetate.
03.011	Benzyl methyl ether		10910 538-86-3	Liquid C ₈ H ₁₀ O 122.17	Practically insoluble or insoluble Freely soluble	169 MS 95 %	1.498-1.504 0.962-0.968	
03.012	Benzyl octyl ether		54852-64-1	Liquid C ₁₅ H ₂₄ O 220.35	Practically insoluble or insoluble Freely soluble	148 (12 hPa) MS 95 %	1.485-1.491 0.903-0.909	
03.015	Ethyl geranyl ether		40267-72-9	Liquid C ₁₂ H ₂₂ O 182.31	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.463-1.469 0.861-0.867	
03.016	Hexyl methyl ether		4291 4747-07-3	Liquid C ₇ H ₁₆ O 116.20	Practically insoluble or insoluble Freely soluble	126 MS 95 %	1.395-1.401 0.766-0.772	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 23, Revision 3

FL-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)	Specification comments
03.020	alpha-Terpinyl methyl ether		14576-08-0	Liquid C ₁₁ H ₂₀ O 168.28	Practically insoluble or insoluble Freely soluble	216 MS 95 %	1.461-1.467 0.885-0.891	Racemate.
03.022	1-Methoxy-1-decene		4161 79930-37-3	Liquid C ₁₁ H ₂₂ O 170	Insoluble Soluble	89 (12 hPa) IR NMR 98 %	1.430-1.438 0.807-0.817	Mixture of (E)- and (Z)- isomers (EFFA, 2010a). > 40 % E isomer and > 40 % Z isomer (EFFA, 2011a).
03.024	Digeranyl ether		4664 31147-36-1	Liquid C ₂₀ H ₃₄ O 290.48	Sparingly soluble Soluble	130 (0.33 Torr) NMR MS >96%	1.477-1.487 0.867-0.876	
04.059	Carvacryl methyl ether		11224 6379-73-3	Liquid C ₁₁ H ₁₆ O 164.25	Very slightly soluble Freely soluble	217 MS 95 %	1.501-1.507 0.937-0.943	
04.067	1-Ethoxy-2-methoxybenzene		17600-72-5	Liquid C ₉ H ₁₂ O ₂ 152.19	Very slightly soluble Freely soluble	213 MS 95 %	1.518-1.524 1.044-1.050	
04.068	1-Ethoxy-4-methoxybenzene		5076-72-2	Solid C ₉ H ₁₂ O ₂ 152.19	Practically insoluble or insoluble Freely soluble	217 37 MS 95 %	n.a. n.a.	
04.069	1-Ethyl-4-methoxybenzene		1515-95-3	Liquid C ₉ H ₁₂ O 136.19	Practically insoluble or insoluble Freely soluble	195 MS 95 %	1.504-1.510 0.955-0.961	
04.075	1-Methoxynaphthalene		2216-69-5	Liquid C ₁₁ H ₁₀ O 158.20	Practically insoluble or insoluble Freely soluble	270 6 MS 95 %	1.622-1.628 1.093-1.099	
04.079	Methyl-4-methoxybenzyl ether		1515-81-7	Liquid C ₉ H ₁₂ O ₂ 152.19	Practically insoluble or insoluble Freely soluble	225 MS 95 %	1.508-1.514 1.023-1.029	Register name to be changed to Methyl 4-methoxybenzyl ether.
04.084	1,2,3-Trimethoxybenzene		634-36-6	Solid C ₉ H ₁₂ O ₃ 168.19	Practically insoluble or insoluble Freely soluble	235 47 MS 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 23, Revision 3

FL-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)	Specification comments
08.127	2-(4-Methoxyphenoxy)propionic acid			Solid C ₁₀ H ₁₂ O ₄ 196.20	Slightly soluble Freely soluble	377 158 NMR 95 %	n.a. n.a.	Racemate (EFFA, 2010a). CASrn to be introduced in Register 158833-38-6.
09.687	2-Phenoxyethyl butyrate		23511-70-8	Liquid C ₁₂ H ₁₆ O ₃ 208.26	Practically insoluble or insoluble Freely soluble	130 (5 hPa) MS 95 %	1.495-1.501 1.057-1.063	
13.200	5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran		4680 1120363-98-5	Liquid C ₁₃ H ₂₆ O 198.35	Insoluble Slightly soluble	93 (10hPa) IR NMR MS 95 %	1.4450- 1.4470 0.8620- 0.8640	Approx. 61 % of (2R,5R,6R)-2,6-Diethyl-5- isopropyl-2- methyltetrahydropyran (DIMP) and (2S,5S,6S)- DIMP; 33 % of (2S,5R,6R)-DIMP and (2R,5S,6S)-DIMP; 4 % of (2R,5R6S)-DIMP and (2S,5S,6R)-DIMP; 1 % of (2S,5R,6S)-DIMP and (2R,5S,6R)-DIMP.

- 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 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) ($\mu\text{g/capita/day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
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)	
03.024	Digeranyl ether		49	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	l-Menthoxyethanol		15	Class III A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) ($\mu\text{g/capita/day}$)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.248	Vanillin 3-(1-menthoxy)propane-1,2-diol acetal		0.61	Class III A3: Intake below threshold	4)	6)	
03.020	alpha-Terpinyl methyl ether		4.1	Class III A3: Intake below threshold	4)	6)	
03.022	1-Methoxy-1-decene		6.1	Class III 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)	
13.200	5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran		12	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) = $\mu\text{g/capita/day}$.

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

- 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.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Hydroxy-1.8-cineole		Not evaluated as flavouring substance		Not in EU-Register
	2-Phenoxyethanol		Not evaluated as flavouring substance		Not in EU-Register
02.224	3-(1-Menthoxy)propane-1,2-diol 1408		No safety concern a)	Class I A3: Intake below threshold	
05.018	Vanillin 889		No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	
08.002	Acetic acid 81		Category 1 d) No safety concern e) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.005	Butyric acid 87		Category 1 d) No safety concern e) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

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

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

a) (JECFA, 2005c).

b) (JECFA, 2002b).

c) (CoE, 1992).

d) (SCF, 1995).

e) (JECFA, 1999b).

ND) Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.014	alpha-Terpineol		3045 62 98-55-5	366 JECFA specification (JECFA, 1998b)	2600	No safety concern a) Category A b)	
03.001	1,8-Cineole		2465 182 470-82-6	1234 JECFA specification (JECFA, 2003b)	1200	No safety concern c) Category B b)	
03.003	Benzyl ethyl ether		2144 521 539-30-0	1252 JECFA specification (JECFA, 2003b)	0.0024	No safety concern c) Deleted b)	
03.005	2-Butyl ethyl ether		3131 10911 2679-87-0	1231 JECFA specification (JECFA, 2003b)	6.9	No safety concern c)	
03.006	2-Methoxyethyl benzene		3198 11812 3558-60-9	1254 JECFA specification (JECFA, 2003b)	26	No safety concern c)	
03.007	1,4-Cineole		3658 11225 470-67-7	1233 JECFA specification (JECFA, 2003b)	3.9	No safety concern c)	
03.010	Benzyl butyl ether		2139 520 588-67-0	1253 JECFA specification (JECFA, 2003b)	0.012	No safety concern c) Deleted b)	
03.019	Prenyl ethyl ether		3777 22094-00-4	1232 JECFA specification (JECFA, 2003b)	21	No safety concern c)	
04.014	1-Methoxy-2-methylbenzene		2680 187 578-58-5	1242 JECFA specification (JECFA, 2003b)	2.4	No safety concern c) Deleted b)	
04.015	1-Methoxy-4-methylbenzene		2681 188 104-93-8	1243 JECFA specification (JECFA, 2003b)	0.49	No safety concern c) Category B b)	
04.016	1,3-Dimethoxybenzene		2385 189 151-10-0	1249 JECFA specification (JECFA, 2003b)	4.6	No safety concern c) Category A b)	
04.032	Anisole		2097 2056	1241 JECFA specification (JECFA, 2003b)	0.024	No safety concern c)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
04.033	beta-Naphthyl ethyl ether		100-66-3 2768 2058 93-18-5	2003b) 1258 JECFA specification (JECFA, 2003b)	43	Category B b) No safety concern c) Category A b)	
04.034	1,4-Dimethoxybenzene		2386 2059 150-78-7	1250 JECFA specification (JECFA, 2003b)	15	No safety concern c) Category A b)	
04.038	Carvacryl ethyl ether		2246 11840 4732-13-2	1247 JECFA specification (JECFA, 2003b)	0.085	No safety concern c)	
04.039	1-Methoxy-4-propylbenzene		2930 11835 104-45-0	1244 JECFA specification (JECFA, 2003b)	20	No safety concern c)	No ADI allocated (JECFA, 1981a).
04.040	1,2-Dimethoxy-4-vinylbenzene		3138 11228 6380-23-0	1251 JECFA specification (JECFA, 2003b)	0.012	No safety concern c)	
04.043	1-Isopropyl-2-methoxy-4-methylbenzene		3436 11245 1076-56-8	1246 JECFA specification (JECFA, 2003b)	1.7	No safety concern c)	
04.054	Isobutyl beta-naphthyl ether		3719 11886 2173-57-1	1259 JECFA specification (JECFA, 2003b)	1.2	No safety concern c)	
04.062	1,2-Dimethoxybenzene		3799 10320 91-16-7	1248 JECFA specification (JECFA, 2003b)	1.6	No safety concern c)	
04.063	1,3-Dimethyl-4-methoxybenzene		3828 6738-23-4	1245 JECFA specification (JECFA, 2003b)	0.12	No safety concern c)	
04.074	2-Methoxynaphthalene		93-04-9	1257 JECFA specification (JECFA, 2003b)	3.5	No safety concern c)	
08.049	Phenoxyacetic acid		2872 2005 122-59-8	1026 JECFA specification (JECFA, 2002d)	30	No safety concern d) Deleted b)	
09.487	2-Phenoxyethyl isobutyrate		2873 2089 103-60-6	1028 JECFA specification (JECFA, 2002d)	1.7	No safety concern d) Deleted b)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.830	Terpineol acetate		3047 205 8007-35-0	368 JECFA specification (JECFA, 1998b)	220	No safety concern a)	
13.037	2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran		3236 2269 16409-43-1	1237 JECFA specification (JECFA, 2003b)	3.8	No safety concern c) Category B b)	
13.088	3,6-Dihydro-4-methyl-2-(2-methylprop-1-en-1-yl)-2H-pyran		3661 1786-08-9	1235 JECFA specification (JECFA, 2003b)	0.85	No safety concern c)	
13.094	2,6,6-Trimethyl-2-vinyltetrahydropyran		3735 10976 7392-19-0	1236 JECFA specification (JECFA, 2003b)	0.024	No safety concern c)	

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

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2000a).

b) (CoE, 1992).

c) (JECFA, 2004a).

d) (JECFA, 2002c).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products⁸ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous⁹ (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

⁸ "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

⁹ "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000a). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 – 15.0

The “normal and maximum use levels” are provided by Industry all 20 candidate substances in the present flavouring group (EFFA, 2003k; EFFA, 2004af; EFFA, 2004j; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2008a; Flavour Industry, 2009g; Flavour Industry, 2010i; Flavour Industry, 2011b) (Table II.1.2).

Table II.1.2. Normal and Maximum use levels (mg/kg) for candidate substances in FGE.23Rev 3 (EFFA, 2003k; EFFA, 2004af; EFFA, 2004j; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2008a; Flavour Industry, 2009g; Flavour Industry, 2010i; Flavour Industry, 2011b)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.24	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
7	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.24	-	2	-	-	5	10	5	5	-	2	-	-	10	-	2	2	-	10
8	-	5	-	-	10	20	20	20	-	10	-	-	20	-	10	10	-	20
03.00	3	2	3	2	-	10	5	10	2	2	-	-	5	15	3	10	-	5
8	15	10	15	10	-	50	25	50	10	10	-	-	25	75	15	50	-	25
03.01	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
1	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
03.01	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
2	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
03.01	3	2	3	3	-	10	5	10	2	2	-	-	5	10	3	10	15	5
5	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25

Table II.1.2. Normal and Maximum use levels (mg/kg) for candidate substances in FGE.23Rev 3 (EFSA, 2003k; EFSA, 2004af; EFSA, 2004j; EFSA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2008a; Flavour Industry, 2009g; Flavour Industry, 2010i; Flavour Industry, 2011b)

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
03.01	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
6	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
03.02	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
0	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
03.02	0,001	0,001	0,01	0,001	0,001	0,1	-	0,01	0,001	0,001	-	-	0,001	-	0,1	0,1	0,001	0,001
2	5	5	5	5	5	5	-	5	5	5	-	-	5	-	5	5	5	5
	0,012	0,012	0,12	0,012	0,012	1,2		0,12	0,012	0,012			0,012		1,2	1,2	0,012	0,012
	5	5	5	5	5	5		5	5	5			5		5	5	5	5
03.02	-	-	50	-	-	70	-	-	-	-	-	-	-	-	15	30	-	50
4	-	-	100	-	-	100	-	-	-	-	-	-	-	-	30	60	-	100
04.05	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
9	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
04.06	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
7	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
04.06	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
8	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
04.06	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
9	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
04.07	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
5	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
04.07	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
9	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
04.08	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
4	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.12	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
7	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
09.68	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
7	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.20	0,5	0,5	0,1	0,5	0,5	1	5	1	-	-	0,5	1	1	-	0,1	0,5	-	-
0	5	10	10	10	10	50	10	5	-	-	5	50	10	-	5	5	-	-

The candidate substances [FL-no. 02.248 and 13.200] are also anticipated to be used in chewing gum, which is not covered by any of the food categories. Normal/maximum use levels for chewing gum are reported to be 10/20 mg/kg for [FL-no: 02.248] and 50/1000 mg/kg for [FL-no:13.200]. For chewing gum, the intake estimate is 2 g/day. It is anticipated that all of the flavouring substance is released from the chewing gum. In the calculation of the mTAMDI of these two candidate substances, use level figures in Table II.1.2 and the use level of chewing gum ((use level of chewing gum in mg/kg) x (2 g daily intake of chewing gum) = mg/person/day) are summed up to a total mTAMDI value of 2500 µg/person/day for [FL-no: 02.248] and 860 µg/person/day for [FL-no: 13.200]. These values are presented in table II.2.3 and 6.1.

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation (EC) No 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for each of 20 flavouring substances in the present flavouring group (EFFA, 2003k; EFFA, 2004af; EFFA, 2004j; EFFA, 2007a; Flavour Industry, 2006a; Flavour Industry, 2008a; Flavour Industry, 2009g; Flavour Industry, 2010i; Flavour Industry, 2011b). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
04.059	Carvacryl methyl ether	3200	Class I	1800
04.084	1,2,3-Trimethoxybenzene	3200	Class I	1800
03.008	2-Acetoxy-1,8-cineole	3500	Class II	540
03.011	Benzyl methyl ether	3200	Class II	540
03.012	Benzyl octyl ether	3200	Class II	540
03.015	Ethyl geranyl ether	3200	Class II	540
03.016	Hexyl methyl ether	3200	Class II	540
03.024	Digeranyl ether	14000	Class II	540
04.079	Methyl-4-methoxybenzyl ether	3200	Class II	540
02.247	1-Menthoxyethanol	3900	Class III	90
02.248	Vanillin 3-(1-menthoxy)propane-1,2-diol acetal	2500	Class III	90
03.020	alpha-Terpinyll methyl ether	3200	Class III	90
03.022	1-Methoxy-1-decene	58	Class III	90
04.067	1-Ethoxy-2-methoxybenzene	3200	Class III	90
04.068	1-Ethoxy-4-methoxybenzene	3200	Class III	90
04.069	1-Ethyl-4-methoxybenzene	3200	Class III	90
04.075	1-Methoxynaphthalene	3200	Class III	90
08.127	2-(4-Methoxyphenoxy)propionic acid	3200	Class III	90
09.687	2-Phenoxyethyl butyrate	3900	Class III	90
13.200	5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran	860	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The candidate substances are examples of aliphatic, alicyclic or aromatic ethers. On the basis of their structure they can be divided into seven subgroups:

- 1) aliphatic ethers [FL-no: 03.015, 03.016 and 03.022],
- 2) alicyclic ethers [FL-no: 03.008 and 13.200],
- 3) alicyclic hydrocarbons with an ether side chain [FL-no: 02.247, 02.248 and 03.020] of which [FL-no: 02.248] has an acetal moiety,
- 4) benzyl ethers [FL-no: 03.011 and 03.012],
- 5) phenol ethers [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687],
- 6) naphthol ethers [FL-no: 04.075] and
- 7) long chain aliphatic ethers [FL-no: 03.024] (see Table III.1).

Table III.1. Subgroups. The supporting substances are listed in brackets.

FL-no	EU Register name	Structural formula
1 Aliphatic Ethers		
03.015	Ethyl geranyl ether
03.016	Hexyl methyl ether
03.022	1-Methoxy-1-decene
(03.005)	(2-Butyl ethyl ether)
(03.006)	(2-Methoxyethyl benzene)
(03.019)	(Prenyl ethyl ether)
2 Alicyclic Ethers		
03.008	2-Acetoxy-1,8-cineole
13.200	5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran
(03.001)	(1,8-Cineole)
(03.007)	(1,4-Cineole)

Table III.1. Subgroups. The supporting substances are listed in brackets.

FL-no	EU Register name	Structural formula
(13.037)	(2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran)
(13.088)	(3,6-Dihydro-4-methyl-2-(2-methylprop-1-en-1-yl)-2H-pyran)
(13.094)	(2,6,6-Trimethyl-2-vinyltetrahydropyran)
3 Alicyclic Hydrocarbons with Ether Side Chain		
02.247	1-Menthoxyethanol
02.248	Vanillin 3-(1-menthoxy)propane-1,2-diol acetal
03.020	alpha-Terpinyl methyl ether
(02.014)	(alpha-Terpineol)
(09.830)	(Terpineol acetate)
4 Benzyl Ethers		
03.011	Benzyl methyl ether
03.012	Benzyl octyl ether
(03.003)	(Benzyl ethyl ether)
(03.010)	(Benzyl butyl ether)
5 Phenol Ethers		
04.059	Carvacryl methyl ether
04.067	1-Ethoxy-2-methoxybenzene
04.068	1-Ethoxy-4-methoxybenzene
04.069	1-Ethyl-4-methoxybenzene
04.079	Methyl 4-methoxybenzyl ether
04.084	1,2,3-Trimethoxybenzene

Table III.1. Subgroups. The supporting substances are listed in brackets.

FL-no	EU Register name	Structural formula
08.127	2-(4-Methoxyphenoxy)propionic acid
09.687	2-Phenoxyethyl butyrate
(04.014)	(1-Methoxy-2-methylbenzene)
(04.015)	(1-Methoxy-4-methylbenzene)
(04.016)	(1,3-Dimethoxybenzene)
(04.032)	(Anisole)
(04.034)	(1,4-Dimethoxybenzene)
(04.038)	(Carvacryl ethyl ether)
(04.039)	(1-Methoxy-4-propylbenzene)
(04.040)	(1,2-Dimethoxy-4-vinylbenzene)
(04.043)	(1-Isopropyl-2-methoxy-4-methylbenzene)
(04.062)	(1,2-Dimethoxybenzene)
(04.063)	(1,3-Dimethyl-4-methoxybenzene)
(08.049)	(Phenoxyacetic acid)
(09.487)	(2-Phenoxyethyl isobutyrate)
6 Naphthol Ethers		
04.075	1-Methoxynaphthalene
(04.033)	(beta-Naphthyl ethyl ether)

Table III.1. Subgroups. The supporting substances are listed in brackets.

FL-no	EU Register name	Structural formula
(04.054)	(Isobutyl beta-naphthyl ether)
(04.074)	(2-Methoxynaphthalene)
7 Long Chain Aliphatic Ether		
03.024	Digeranyl ether

No toxicokinetic studies were found on the candidate substances. Data on absorption, distribution and excretion are available for supporting substances and are described below.

III.2. Absorption, Distribution and Elimination

Subgroup 1: Aliphatic Ethers [FL-no: 03.015, 03.016 and 03.022]

The aliphatic ethers are expected to be rapidly absorbed from the gastrointestinal tract and excreted.

Data on absorption, metabolism and urinary excretion of the aliphatic ethers in animals are available for the structurally-related substance methyl tertiary-butyl ether (MTBE). MTBE is completely absorbed, metabolised and excreted following oral administration of 40 mg/kg bw to rats. MTBE was totally absorbed by the Gastrointestinal (GI) tract (as demonstrated by the identical Area Under the Curve (AUC) value calculated after oral and intravenous administration); the peak plasma concentration was reached within 15 minutes. It was then rapidly eliminated from the blood, with a reported half-life of 30 minutes, by exhalation as such (> 60 % of the administered dose) and metabolism to tertiary-butyl alcohol, which is mainly excreted into the urine (blood half-life = 1-2 hours). At a higher dose (400 mg/kg bw), metabolism was saturated and the proportion of renal [¹⁴C] excretion decreased relatively to the pulmonary route of elimination. At 48 hours post exposure, almost all the administered radioactivity was eliminated at both doses. No tissue-specific affinity or gender differences were described in the toxicokinetics and distribution of MTBE (Miller et al., 1997).

Subgroup 2: Alicyclic Ethers [FL-no: 03.008 and 13.200]

After the oral administration of 200 mg/kg bw 1,8-cineole [FL-no: 03.001] (synonym: eucalyptol) to rabbits, peak plasma concentration of the parent compound occurred within 30 minutes and reached a maximum plasma level of 840 µg/dl, while the plasma level of the principal unconjugated metabolite, (+)-2-endo-hydroxy-1,8-cineole, peaked at 2400 µg/dl within one hour post exposure and then decreased slowly between two and six hours. Peak plasma levels (1250 µg/dl) of the major conjugated metabolite, the glucuronide of (+)-2-exo-hydroxy-1,8-cineole, occurred within 1.5 to 2 hours after dosing (Miyazawa et al., 1989).

When 4, 20 or 40 µl of rosemary oil as a oil/water emulsion, containing 39 % 1,8-cineole (approximately equivalent to 52, 260, and 520 mg/kg bw of 1,8-cineole, respectively) were administered orally to mice, blood levels of the parent compound reached a peak level 5 minutes following the exposure. At 260 mg/kg bw, blood levels remained fairly constant over the following 90 minutes. At 520 mg/kg bw, the peak blood concentration dropped to 60 % of the maximum value and remained in that range for the following 80 minutes (Kovar et al., 1987). These results indicate that at doses up to 200 mg/kg, 1,8-cineole is rapidly

absorbed into the blood, and eliminated by conjugation to polar metabolites. At higher doses, metabolism appears to be slower, due to saturation of the metabolic pathway.

In humans, data are available for the inhalation route. In four healthy volunteers exposed for twenty minutes to air passing over 4 ml of 1,8-cineole via a closed breathing circuit, 1,8-cineole showed biphasic elimination from the blood. The peak blood concentration was reached within 15 minutes in all the subjects, attaining similar values (about 460 - 1100 ng/ml), indicating small interindividual differences in the absorption phase. The mean half-life for distribution was 6.7 minutes, whereas the half-life for elimination is 104.6 minutes. However, 1,8-cineole distribution seemed to be affected by the body composition of the volunteer (Jäger et al., 1996).

Subgroup 3: Alicyclic Hydrocarbons with Ether Side Chain [FL-no: 02.247, 02.248 and 03.020]

Terpenes are rapidly absorbed in the GI tract and due to their lipophilicity are extensively distributed in the body. Using radio-labelled citral (3,7-dimethyl-2,6-octadienal), it was shown that in rats, ^{14}C is widely distributed within 72 hours and citral is probably metabolised through various pathways to common biological metabolites which are incorporated into tissues. This however, constitutes only a very small proportion of the dose administered. Following oral administration of citral in rats, 67 % of the 5 mg/kg bw dose was excreted within 24 hours, 45 % of this was excreted in the urine. Radio-labelled metabolites appeared within 2 hours of administration (Diliberto et al., 1988).

Following administration of 5 mg/kg bw of citral in rats, 95 % was excreted within 24 hours. The majority of this dose (60 %) was excreted in the urine, 20 % was excreted in the lungs as $^{14}\text{CO}_2$ and 17 % was excreted in the faeces. A very small proportion (0.5 %) of ^{14}C found in the urine was found to be unchanged citral and 1.5 % of the total ^{14}C was retained in the liver. At higher levels (960 mg/kg bw), between 60 % and 70 % of the ^{14}C was excreted within 24 hours. Again, this was mostly excreted in the urine (47 %). However, a lower level was excreted as $^{14}\text{CO}_2$. Faecal excretion was much more delayed than at lower doses up to 36 hours after administration and the rate of faecal elimination increased between 36 and 96 hours following administration. 95 % of the total ^{14}C was eliminated within 96 hours. The identities of the metabolites were not determined in this study, however, they were shown to be polar hexane-insoluble unsaturated compounds (Phillips et al., 1976).

Subgroup 4: Benzyl Ethers [FL-no: 03.011 and 03.012]

Aromatic ethers containing a benzene ring are thought to be absorbed, distributed and excreted in a similar way as alkylbenzenes.

On administration of n-propylbenzene to chinchilla rabbits, approximately 60 - 70 % was excreted in the urine within 24 hours as conjugates of glucuronide and hippuric acid. Ethylphenylcarbonyl glucosiduronic acid was readily isolated from the urine of these rabbits (El Masry et al., 1956). In the same study, about 50 % of the administered dose of butylbenzene was shown to be excreted as the glucuronides of methyl-2-phenylethylcarbinol and phenylpropylcarbinol and around 20 % was excreted as phenaceturic acid. Approximately 4 % was excreted as hippuric acid.

Following administration of 5 ml/kg bw of n-propylbenzene, the urinary sulphate ratio of inorganic/total sulphate was reduced considerably between 24 and 72 hours. The sulphate ratio returned to normal after 96 hours. An increase in alkyl chain length was shown to be associated with an increase in the time taken for the sulphate ratio to return to normal, which indicates an increase in the time taken to eliminate the substance from the body (Gerarde and Ahlstrom, 1966).

Alkylbenzenes appear to be metabolised to innocuous products and excreted as conjugates of glucuronides and as hippuric acid in the urine.

Subgroup 5: Phenol Ethers [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687]

When the supporting substance anisole [FL-no: 04.032] (500 mg/kg bw) was administered to rabbits via stomach tube, about 80 % of the dose was excreted in the urine within 24 hours, mainly as the products of p-hydroxylation and the corresponding glucuronide and sulphate. The unchanged ethers were not detected in the urine and no smell of anisole was detected in the breath of the rabbits (Bray et al., 1953).

The majority (up to about 70 %) of p-methylanisole administered via gavage to six rabbits was excreted in the urine within 24 hours, after oxidation to anisic acid and subsequent glucuronidation and as p-cresol (the latter metabolite accounting for about 27 % of the administered dose) (Bray et al., 1955).

When a single dose of [^{14}C]-p-propylanisole (labelled at the methoxy position) was administered to female Wistar albino rats via oral intubation and to male CD-1 mice via the intraperitoneal route at dose levels of 0.05, 0.5, 5, 50, 500 or 1500 mg/kg bw, at the lowest dose level the majority of radioactivity was excreted as $^{14}\text{CO}_2$ in the expired air (81.6 and 74.5 % in rats and mice, respectively), whereas the urinary excretion accounted for 8.0 and 15.0 % in rats and mice, respectively. As dose levels increased up to 1500 mg/kg bw, approximately equal amounts were excreted in the urine (37.1 and 38.0 % in rats and mice, respectively) and in the expired air as $^{14}\text{CO}_2$ (47.2 and 49.9 % in rats and mice, respectively) within 72 hours, suggesting that the O-demethylation pathway becomes saturated at the higher dose levels. Approximately 1 - 5 % was excreted in the faeces regardless of the administered dose. However, in all the experimental conditions tested, more than 80 % of the administered dose was excreted within 72 hours after the treatment (Sangster et al., 1983).

Approximately 67 % of a 100 μg dose of [^{14}C]-methoxy-labelled p-propylanisole administered by gelatin capsule to two humans was recovered within 8 hours, the majority of which (43 %) in the expired air and the remainder (about 24 %) in the urine (Sangster et al., 1987).

Subgroup 6: Naphthol Ethers [FL-no: 04.075]

After absorption, the supporting substance beta-naphthyl methyl ether [FL-no: 04.033] is hydroxylated and excreted as a glucuronide (Williams, 1959a).

Subgroup 7: Long Chain Aliphatic Ether [FL-no: 03.024]

No data are available on the absorption, distribution and excretion of the substance in this sub-group or any supporting substances but as with the other substances in this flavouring group, digeranyl ether [FL-no: 03.024] would be expected to be rapidly absorbed from the gastrointestinal tract and excreted as polar metabolites in the urine and in the exhaled air as CO_2 in a similar way to the aliphatic saturated tertiary alcohols found in FGE.18, Revision 1 (EFSA, 2009a) and the non-polar hydrocarbons found in FGE.25 (EFSA, 2008ba).

III.3. Metabolism

No metabolism studies were found for the candidate substances.

Several metabolic options are available to aliphatic and aromatic ethers. One pathway for aliphatic and aromatic ethers is O-dealkylation to form the corresponding aldehydes and alcohols if a suitable alkyl substituent (methyl or ethyl) is attached to the ether oxygen. The resulting alcohols may be further oxidised followed by conjugation and excretion, while the aldehydes (i.e. acetaldehyde and formaldehyde) are oxidised to carboxylic acids, which participate in fundamental biochemical pathways, including the fatty acid pathway and tricarboxylic acid cycle, resulting in CO_2 expiration. Alternatively, the aliphatic acyclic or

aromatic moiety may undergo cytochrome P450-catalysed C-oxidation (ring-hydroxylation or side-chain oxidation), followed by conjugation with sulphate or glucuronic acid and then excretion, mainly via the urinary route.

Subgroup 1: Aliphatic Ethers [FL-no: 03.015, 03.016 and 03.022]

Aliphatic ethers included in the present evaluation are expected to undergo NADPH-dependent P450-catalysed O-dealkylation to the corresponding alcohols and aldehydes.

In vitro, by using liver microsomes from Sprague-Dawley rats, methyl tertiary-butyl ether (MTBE) was O-demethylated to form almost equal amounts of t-butyl alcohol (TBA) and formaldehyde (Brady et al., 1990). MTBE metabolism was inhibited 35 % by monoclonal antibodies to CYP2E1 and increased in acetone-induced microsomes, indicating that MTBE is partially metabolised by this P450 isozyme. Pretreatment of rats via intraperitoneal injection with 1 or 5 ml MTBE/kg bw did not affect the activity of CYP2E1, but induced CYP2B1, suggesting that this CYP may be also involved in MTBE biotransformation (Brady et al., 1990).

In vivo, oral administration of 40 mg/kg bw of MTBE to rats resulted in the rapid production of TBA. The alcohol was detected in the blood (half life = 1-2 hours) and in the urine. At a 10-fold higher dose, the metabolism appeared to be saturated, and a higher percentage of the administered dose was recovered as parental compound in the exhaled air (Miller et al., 1997).

In other studies methyl tertiary-butyl ether (MTBE) and ethyl tertiary-butyl ether were administered to rat by inhalation, while TBA, the major initial metabolite of the two ethers, was studied after oral gavage (250 mg/kg bw). Only minor amounts of TBA and its conjugate were detected in the urine of rats dosed with the ethers, whereas 2-methyl-1,2-propanediol, 2-hydroxyisobutyrate and an unidentified TBA conjugate were the major urinary metabolites. The same major metabolites were found in TBA-treated rats, being unchanged TBA, its glucuronide and acetone only detected in minor amounts. Similar results were obtained in one human volunteer, taking 5 mg/kg TBA orally, suggesting that TBA, formed by MTBE and ETBE, is extensively metabolised by further oxidation reactions (Bernauer et al., 1998).

It has been reported that diethyl ether was biodegraded to $^{14}\text{CO}_2$ in amounts of 1 to 5 % in rats (Krantz and Carr, 1969), due to its O-demethylation to ethanol and acetaldehyde, followed by oxidation to acetate, which eventually enters the citric acid cycle. This further supports the likelihood that the candidate aliphatic ethers may undergo O-dealkylation to form their corresponding alcohols and aldehydes, which are expected to subsequently participate in the fatty acid pathway and tricarboxylic acid cycle.

The anticipated demethylated product of 1-methoxy-1-decene [FL-no: 03.022] is an enol, which will rearrange to the aldehyde, which subsequently can be oxidised to the carboxylic acid.

Subgroup 2: Alicyclic Ethers [FL-no: 03.008 and 13.200]

In humans and other animals, alicyclic ethers, such as the supporting substances 1,4-cineole [FL-no: 03.007] and 1,8-cineole [FL-no: 03.001], have been shown to be oxidised via P450 isoenzymes to yield polar hydroxylated metabolites, which are conjugated and excreted or further oxidised and excreted. Cleavage of the ether is, at most, a very minor metabolic pathway (Hiroi et al., 1995; Miyazawa et al., 2001a; Miyazawa et al., 2001b; Miyazawa and Shindo, 2001).

The metabolism of 1,8-cineole [FL-no: 03.001] has been studied in various animal species. It has been reported that 1,8-cineole principally undergoes ring-hydroxylation to form 2- or 3-hydroxy-1,8-cineole, which are subsequently excreted as the glucuronic acid conjugates (Williams, 1959a) (see Figure III.1). Indeed, following the gavage administration of 800 mg 1,8-cineole/kg bw to male albino rats, major metabolites included 2- and 3-hydroxy-1,8-cineole and their conjugates and 1,8-dihydroxy-10-carboxy-p-

menthane, which were hypothesised to be formed by the oxidation of the metabolite p-menthane-1,8-diol formed by cleavage of the ether linkage (Madyastha and Chadha, 1986).

Figure III.1 Metabolism of 1,8-cineole (eucalyptol) in rats and humans.

These results are consistent with a more recent study, in which 1,8-cineole [FL-no: 03.001] metabolism in microsomes from male Hooded Wistar rats and humans was studied (Pass et al., 2001). To determine the effect on the efficiency of biotransformation, expressed as the intrinsic clearance ($V_{\max}/K_m$) of the reaction, caused by possible induction of metabolism, rats were pre-treated daily for six days with a mixture of terpenes (255 mg 1,8-cineole/kg bw; 4 mg p-cymene/kg bw; 34 mg limonene/kg bw; 103 mg α -pinene/kg bw) by gavage, or 80 mg/kg bw of phenobarbital (PB). Liver microsomes prepared from pre-treated and control rats, as well human liver microsomes pooled from seven male patients were incubated with 5 – 200 μ M 1,8-cineole. Intrinsic clearance values were as follows: 27.5; 258.2; 1824.7 and 11.6 μ l-mg protein⁻¹-minute⁻¹ in microsomes from control, terpene-treated, PB-treated rats and humans, respectively. The efficiency in 1,8-cineole metabolism was similar in control rat and human microsomes, whereas terpenes and, at a higher extent, PB-induced rat microsomes metabolised 1,8-cineole more efficiently. This result suggests that terpenes are able to induce their own metabolism, in which CYP2B1 (induced by PB) is very likely involved (Pass et al., 2001). Although with differences in their relative amounts, qualitatively the various liver microsomes produced the same hydroxylated metabolites. Control rat microsomes produced 3-hydroxy-1,8-cineole as the major metabolite, followed by 2- and 9-hydroxycineole. Microsomes from terpene-treated rats produced similar amounts of 2- and 3-hydroxy-1,8-cineole and lesser amounts of 9-hydroxy-1,8-cineole. Of the six metabolites detected in the microsomes from PB-treated rats, 2-hydroxy-1,8-cineole was the major metabolite, followed by 3- and 9-hydroxy-1,8-cineole, whereas the remaining three metabolites consisted of trace amounts of 7-hydroxy-1,8-cineole, 9-cineolic acid and one unknown hydroxycineole metabolite. 2-Hydroxy-1,8-cineole was the major metabolite from pooled human liver microsomes, while 9-hydroxy-1,8-cineole was the minor metabolite. The authors concluded that in rats and humans, oxidation was preferred at the aliphatic ring carbons over methyl substituents (Pass et al., 2001).

The metabolism of 1,8-cineole [FL-no: 03.001] was studied *in vivo* in rabbits treated by gavage with 200 mg/kg bw. The major metabolites were identified as 2- and 3-hydroxy-1,8-cineole (Miyazawa et al., 1989). When rat and human liver microsomes and recombinant human CYPs (i.e. c-DNA expressed in insect cells) were incubated *in vitro* with 1,8-cineole, it was oxidised at high rates to 2-exo-hydroxy-1,8-cineole (see Figure III.1) (Miyazawa et al., 2001b; Miyazawa and Shindo, 2001). As indicated by results obtained with recombinant CYPs, P450 inducers (PB and pregnenolone 16- α -carbonitrile), and specific P450 inhibitors, the reaction in humans is mainly catalysed by CYP3A2 and 3A4 in rat and human liver microsomes, respectively (Miyazawa et al., 2001a; Miyazawa et al., 2001b). Earlier studies also indicate that CYP3A family is induced by 1,8-cineole. Hepatic microsomes prepared from male Sprague-Dawley rats intraperitoneally injected 300 mg 1,8-cineole/kg bw once a day for five days showed increased levels of 2B1 and 3A2 expression and in their related enzymatic activities (Hiroi et al., 1995).

Hepatic microsomes from beta-naphthoflavone- or PB-pretreated female Wistar rats were used to investigate the inhibitory effects of 1,8-cineole [FL-no: 03.001] on the marker activities of CYP1A1 (ethoxyresorufin-O-deethylase, EROD), 1A2 (methoxyresorufin-O-demethylase, MROD), and 2B1 (pentoxyresorufin-O-dephenylase, PROD). 1,8-Cineole caused no or negligible inhibition on EROD and MROD (up to 150 μ M), while CYP2B1 activity was decreased in the presence of 1,8-cineole. The competition with the specific probe substrate for CYP2B1 indicates that also this isoform is involved in 1,8-cineole metabolism in the rat (De-Oliveira et al., 1999).

Oxidation of 1,4-cineole [FL-no: 03.007] was studied in rat and human liver microsomes as well as with recombinant human CYPs; in all cases the major identified metabolite was 2-exo-hydroxy-1,4-cineole.

Based on the results obtained with single recombinant isoforms on the effects of specific CYP inhibitors and antibodies and on the data from correlation studies, CYP3A4 was identified as the CYP, mainly responsible for 1,4-cineole oxidation. Similarly, CYP3A2 was active in the rat (Miyazawa et al., 2001a).

In rabbits, 1,4-cineole [FL-no: 03.007] is metabolised by ring- and side-chain hydroxylation. Urinary metabolites collected over three days following administration of 10,000 mg 1,4-cineole/rabbit include the ring-hydroxylation product 3,8-dihydroxy-1,4-cineole, the side-chain hydroxylation product 9-hydroxy-1,4-cineole and its corresponding carboxylic acid, 1,4-cineole-9-carboxylic acid. Other metabolites included 8,9-dihydroxy-1,4-cineole and 1,4-cineole-8-en-9-ol. No evidence of ether cleavage was observed at this dose level (Asakawa et al., 1988).

Subgroup 3: Cyclic Hydrocarbons with Ether Side Chain [FL-no: 02.247, 02.248 and 03.020]

The available data on alpha-terpineol [FL-no: 02.014] as supporting substance for subgroup 3, suggest that the candidate substance [FL-no: 03.020] would be metabolised by P450 isoenzymes to yield polar hydroxylated metabolites, which are conjugated to form glucuronic acid conjugates and excreted or are further oxidised and excreted. Cleavage of the ether is a minor metabolic pathway (JECFA, 1999a). The JECFA considered the metabolism of alpha-terpineol at their 51st meeting and they concluded that in humans and animals, terpenoid tertiary alcohols, of which alpha-terpineol is one, are conjugated primarily with glucuronic acid and are excreted in the urine and faeces. Unsaturated terpenoid alcohols may undergo allylic oxidation to form polar diol metabolites, which may be excreted either free or conjugated (Williams, 1959a; Parke et al., 1974a; Parke et al., 1974b; Horning et al., 1976; Ventura et al., 1985).

Male albino rats were administered orally alpha-terpineol [FL-no: 02.014] (600 mg/kg bw), once daily for 20 days as a suspension (in 1 % methyl cellulose solution, 2 ml as final volume). The urinary metabolites were qualitatively identified. A significant amount of the test item was excreted unmetabolised, and the allylic methyl oxidation was the major route of biotransformation. The reduction of the endocyclic double-bond was also seen. In addition, the treatment resulted in a substantial induction of P450-related activities in the liver (Madyastha and Srivatsan, 1988b).

Citral was shown to undergo first pass liver metabolism and also be metabolised by intestinal bacteria. Excretion of metabolites via the bile into the intestine, results in enterohepatic recirculation of citral-derived radioactivity (Diliberto et al., 1988).

Citral has been shown to be metabolised to 7-carboxy-3-methylocta-2,6-dienoic acid and 7-carboxy-3-methylocta-6-enoic acid (Williams, 1959a). These are considered to be innocuous products.

At low pH similar to that found in the stomach, vanillin 3-l-menthoxypropane-1,2-diol acetal [FL-no: 02.248] is readily hydrolysed. In a hydrolysis study, 12 - 39 mM vanillin 3-l-menthoxypropane-1,2-diol acetal underwent 91 % hydrolysis at pH 2 within 45 minutes. At pH 3, approximately 86 % of vanillin 3-l-menthoxypropane-1,2-diol acetal hydrolysed within 90 minutes. At a pH of 4, approximately 92 % of the acetal hydrolysed within 8 hours. At a pH of 5, approximately 12 % of the flavouring agent hydrolysed within 8 hours (Reitz, 1995).

Under acidic conditions, pH 2.6, vanillin propylene glycol acetal began to hydrolyse immediately with approximately 3 % of the acetal disappearing and 92 % hydrolysed within two hours. At a pH of 1.8, approximately 90 % of vanillin propylene glycol acetal hydrolysed immediately and 93 % hydrolysed within five minutes (Bennett, 1997).

As shown above vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] is shown to be hydrolysed to the corresponding ether and vanillin. It is expected that the alcohol groups in this ether subsequently are oxidised and that the carboxylic acid(s) are then excreted as such or after conjugation.

Similarly, l-menthoxy-ethanol [FL-no: 02.247] is anticipated to be oxidised to the corresponding carboxylic acid and excreted.

Subgroup 4: Aromatic Ethers Containing a Benzene Ring [FL-no: 03.011 and 03.012]

No data are available on the candidate substances, however, alkylbenzenes are considered to be metabolised in a similar way to benzyl ethers. Male albino rats were dosed with quantities of n-propylbenzene and other alkylbenzenes at doses of up to 5 ml/kg. After dosing the animals were kept in metabolism cages and urine was collected in 24 hours fractions for 96 hours. Urine samples were analysed for organic and inorganic sulphates. The change in urinary sulphate ratio, inorganic/total was used to ascertain whether ring-hydroxylation had occurred after administration of increasing doses of alkylbenzenes. Following dosing with n-propylbenzene, it was shown that at 48 hours the peak level of excretion is reached and all of the n-propylbenzene is excreted by 96 hours following dosing at 5 ml/kg bw. The authors conclude that alkylbenzenes with shorter side chains are primarily exhaled as unchanged hydrocarbon. As the side chain increases in size, the proportion of alkylbenzene that is ring-hydroxylated and excreted in the urine also increases. Organic sulphate in the urine indicates that ring-hydroxylation is occurring in the body to form phenol derivatives. The longer the alkyl side-chain, the greater the level of organic sulphate in the urine and therefore the greater the level of ring-hydroxylation and production of phenol derivatives (Gerarde and Ahlstrom, 1966).

Following administration of n-propylbenzene, it was observed that the main product in the urine was ethylphenylcarbinyl glucosiduronic acid. This shows that the major route of metabolism is via ethylphenylcarbinol, which is then conjugated with glucuronic acid. Another route is via benzylmethylcarbinol, which is more readily converted to hippuric acid. Another possible route is via omega-oxidation to beta-phenylpropionic acid, which can be subsequently beta-oxidised to benzoic acid (El Masry et al., 1956).

Subgroup 5: Aromatic Ethers Containing a Phenol Ring [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687]

The supporting aromatic ethers are metabolised by ring-hydroxylation, cleavage of the methyl ether (O-demethylation), and/or oxidation of the ring substituents.

Several studies have demonstrated that anisole [FL-no: 04.032] principally undergoes P450-catalysed ring-hydroxylation preferentially at the *p*-position, with O-demethylation and *o*-hydroxylation as the minor pathways (Daly and Jerina, 1969; Daly, 1970; Ohi et al., 1992; Takahara et al., 1986).

Fifteen minutes *in vitro* incubation of 25 - 50 µmoles of 2-2H-anisole with 3-methylcholanthrene (3MC)-induced liver microsomes resulted in *o*- and *p*-hydroxylation to form 2- and 4-hydroxyanisole, respectively (Daly and Jerina, 1969). In a similar study, 50 µmoles anisole [FL-no: 04.032] incubated for 15 minutes with liver homogenates from 3MC-pretreated rats, gave rise to 4 µmoles *p*-hydroxyanisole, 0.8 µmoles *o*-hydroxyanisole and 0.2 µmoles phenol. Thus, in hepatic 3MC induced rat microsomes, *p*-hydroxylation is the major metabolic pathway of anisole biotransformation, while O-demethylation and *o*-hydroxylation are the minor ones (Daly, 1970). In the same study, it is reported that ortho-substituents, including methyl groups, greatly reduced the O-demethylation but had little effects on para-hydroxylation. The presence of a methyl group in meta position had little effects on ortho- and para-hydroxylation of anisole. Para-substitution in the anisole molecule blocked para-hydroxylation of the ring and markedly stimulate O-demethylation and to a lesser extent meta-hydroxylation (Daly, 1970). These features are related to 3MC-induced rat microsomes, since in hepatic microsomes from control rat and rabbit, para-hydroxylation and O-demethylation are almost equally efficient (Daly, 1970).

The effect of oxygen concentration on the metabolism of anisole [FL-no: 04.032] was investigated in a more recent study (Takahara et al., 1986). When phenobarbital-induced rat liver microsomes were incubated with 2 mM anisole, in normoxic conditions, comparable levels of phenol (the product of O-demethylation) and the aromatic hydroxylated products, *p*-hydroxyanisole and *o*-hydroxyanisole were detected in 10 minutes' incubations; no *m*-hydroxylation took place. In hypoxic conditions the levels of the 3 metabolites were markedly decreased. When anisole was incubated for 1 hour at different oxygen concentrations (24, 34, 54, 74, 113 or 223 µM), the formation rates and the relative amounts of metabolites were dependent on the oxygen concentration, as the amount of O-demethylated product started decreasing at oxygen concentrations below 60 µM (typical oxygen pressure in the liver was 35 µM) (Takahara et al., 1986). Results were confirmed in a second study using the same protocol, in which also iso-propoxybenzene metabolism was investigated. Although the dependence on pO₂ slightly differs with the two compounds, in both case the main metabolites were due to *p*-hydroxylation and O-demethylation with lower formation of the *o*-hydroxylation product. No *m*- or side chain hydroxylation products were detected (Ohi et al., 1992).

In vivo experiments on anisole [FL-no: 04.032] confirm that ring-hydroxylation predominates over O-demethylation. Urine collected 24 hours post-administration of 0.5 g/kg bw of anisole via gavage to rabbits revealed that 2 % of the dose was unconjugated *p*-methoxyphenol (major) and, to a lower extent *o*-methoxyphenol, 48 % was conjugated with glucuronic acid, and 29 % was conjugated with sulphate. No evidence of ether cleavage was detected (Bray et al., 1953). The limited ether cleavage in the rabbit was confirmed by a study with rabbit liver microsomes incubated with 2 µmoles anisole for one hour (Axelrod, 1956).

p-Methylanisole [FL-no: 04.015] administered at 700 mg/rabbit to six rabbits via gavage undergoes mainly methyl group oxidation to yield anisic acid (*p*-methoxybenzoic acid), excreted as the glucuronic acid conjugate in the urine. A smaller amount (27 %) of *p*-methylanisole is demethylated and excreted in the urine as the sulphate or glucuronic acid conjugate of *p*-cresol. In humans and dogs, anisic acid (*p*-methoxybenzoic acid) is excreted as conjugates of glucuronic acid and glycine (Bray et al., 1955).

The majority of [¹⁴C]-*p*-propylanisole labelled at the methoxy position is metabolised via O-demethylation, resulting in the expiration of ¹⁴CO₂, α - and ω -1 oxidation of the side-chain, leading to 1'- and 2'-hydroxy-*p*-propylanisole, excreted in the urine and side-chain degradation yielding *p*-methoxyhippuric acid found in the urine conjugated with glycine (see Figure III.2). Radiolabeled *p*-propylanisole was administered to groups of

female Wistar albino rats via oral intubation and to male CD-1 mice via the intraperitoneal route at dose levels of 0.05, 0.5, 5, 50, 500 or 1500 mg/kg bw for both species. The excretion pathway greatly varied with dose. At the lowest dose level (0.05 mg/kg bw/day), the majority of the radioactivity is excreted as $^{14}\text{CO}_2$ in expired air (81.6 and 74.5 % in rats and mice, respectively) compared to urinary excretion (8.0 and 15.0 % in rats and mice, respectively) within 72 hours. At the low dose levels, the major urinary metabolites were *p*-methoxyhippuric acid and 2'-hydroxy-*p*-propylanisole. As dose levels increased, a metabolic shift to α - and ω -1 hydroxylation occurred, yielding greater amounts of the glucuronic acid urinary conjugates of 1'- and 2'-hydroxy-*p*-propylanisole and the side-chain degradation product, *p*-methoxybenzoic acid conjugated with glycine. At the highest dose levels (500 or 1500 mg/kg bw) 1'- and 2'-hydroxy-*p*-propylanisole were present in the urine also as unconjugated products. Based on this study and other available studies in literature on structurally related substances such as trans-anethole (*p*-propenylanisole) and estragole, the plausible metabolic routes of *p*-propylanisole in rats and mice are presented in Figure III.2 (Sangster et al., 1983).

Figure III.2 Metabolism of *p*-propylanisole in rats and humans.

At low doses, the O-demethylation pathway for the metabolism of *p*-propylanisole predominates in humans as well. In a study in humans, two male volunteers were administered a gelatin capsule containing 100 μg [^{14}C]-*p*-propylanisole (1.5 $\mu\text{g/kg}$ bw). The majority (42.7 %) of the radioactivity was accounted for as exhaled $^{14}\text{CO}_2$ within 48 hours, demonstrating that O-demethylation was the principal metabolic pathway for *p*-propylanisole. Comparison with results obtained with anethole and estragole, clearly indicates that *p*-propylanisole, which has a saturated side-chain, is more efficiently and extensively O-demethylated than its two unsaturated congeners. The principal metabolic products identified in the urine included the glycine conjugate of 4-methoxybenzoic acid and the glucuronides of 1- and 2-hydroxy-*p*-propylanisoles and 1,2-dihydroxy-*p*-propylanisoles (Sangster et al., 1987).

p-Dimethoxybenzene [FL-no: 04.034] administered via gavage at 700 mg/kg bw to rabbits undergoes extensive O-demethylation to *p*-methoxyphenol (34 %) followed by excretion as a glucuronic acid or sulphate conjugate. Trace amounts of hydroquinone were reported. O-Demethylation of *p*-dimethoxybenzene was also reported to occur *in vitro* with rabbit liver slices (Bray et al., 1955).

2-(4-Methoxyphenoxy)propionic acid [FL-no: 08.127] is expected to be excreted as such or after conjugation.

Subgroup 6: Naphthol Ethers [FL-no: 04.075]

No metabolism studies were found for the candidate substance, but data are available on the supporting substance 2-methoxynaphthalene. 2-Methoxynaphthalene is excreted as a glucuronic acid conjugate with the methyl ether linkage intact. The exact position of the glucuronic acid residue on the naphthyl moiety was not identified (Williams, 1959a).

Subgroup 7: Long Chain Aliphatic Ethers [FL-no: 03.024]

Whilst no metabolism data have been found for the candidate substance in subgroup 7, digeranyl ether [FL-no: 03.024], data are available on substances that are supporting for the metabolism of longer chain ethers. Tsuji et al., concluded that dealkylation of ethers becomes less likely as the chain length increases and that ω -oxidation is more likely to occur (Tsuji et al., 1978).

Male IISc rats were given [1-3H] geraniol in daily doses of 800 mg/kg bw by gavage for 20 consecutive days. Five urinary metabolites were identified via two primary pathways. In one pathway, the alcohol is oxidised to yield geranic acid (3,7-dimethyl-2,6-octadienoic acid) which is subsequently hydrated to yield 3,7-dimethyl-3-hydroxy-6-octenoic acid (3-hydroxy citronellic acid). In a second pathway, the alcohol undergoes selective ω -oxidation of the C8-methyl to yield 8-hydroxygeraniol and 8-carboxygeraniol, the latter of which undergoes further oxidation to the principal urinary metabolite 2,6-dimethyl-2,6-octadienedioic acid. It was demonstrated that administration of geraniol at a dose of 600 mg/kg bw by gavage for 1, 3 or 6 days induced expression of rat liver microsomal cytochrome P450 and geraniol hydroxylation, but not the activities of rat liver microsomal cytochrome b5, NADPH-cytochrome c reductase, and NADH-cytochrome c reductase, nor the activities of these enzymes in rat lung microsomes. Rabbits are also capable of ω -oxidation of geraniol, as both the Hildebrandt acid and its dihydro form (2,6-dimethyl-2-octendioic acid; reduced or dihydro-Hildebrandt acid) were isolated from the urine of treated animals (Fischer and Bielig, 1940; Asano and Yamakawa, 1950). In both rabbits and rats, the ω -hydroxylation is mediated by the cytochrome P450 system and requires NADPH and oxygen. It has been demonstrated that not only rat liver microsomes are capable of ω -hydroxylating geraniol, but also rat lung and kidney microsomes (JECFA, 2004b).

Unlike geraniol, digeranyl ether does not have free hydroxyl groups to facilitate other routes of biotransformation, therefore this would increase the likelihood that ω -oxidation will occur.

It would be anticipated that following ω -oxidation the resulting metabolites will undergo glucuronidation and be excreted in the urine in a similar way to the aliphatic saturated tertiary alcohols found in FGE.18, Revision 1 (EFSA, 2009a) and the non-polar hydrocarbons found in FGE.25Rev2 (EFSA, 2011k).

III.4. Summary and Conclusions

The candidate substances are aliphatic, alicyclic or aromatic ethers. On the basis of their structures they can be divided into seven subgroups: 1) aliphatic ethers [FL-no: 03.015, 03.016 and 03.022], 2) alicyclic ethers [FL-no: 03.008 and 13.200], 3) alicyclic hydrocarbons with an ether side chain [FL-no: 02.247, 02.248 and

03.020], 4) benzyl ethers [FL-no: 03.011 and 03.012], 5) phenol ethers [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687], 6) naphthol ethers [FL-no: 04.075] and 7) long chain aliphatic ethers [FL-no: 03.024].

No data on absorption, distribution, metabolism or elimination are reported for the 20 candidate substances.

According to the available data on supporting substances, the aliphatic ethers in subgroup 1 [FL-no: 03.015, 03.016 and 03.022], the cyclic ethers in subgroup 2 [FL-no: 03.008 and 13.200], the cyclic hydrocarbon with ether side chain in subgroup 3 [FL-no: 02.247, 02.248 and 03.020], the benzyl ethers in subgroup 4 [FL-no: 03.011 and 03.012] and the phenolic ethers in subgroup 5 [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687] and the long chain aliphatic ether in subgroup 7 [FL-no: 03.024] are all expected to be rapidly absorbed from the gastrointestinal tract and excreted in the exhaled air as CO₂ and as polar metabolites in the urine.

After absorption, the supporting substance, beta-naphthyl methyl ether [FL-no: 04.033], representative for the ether in subgroup 6, 1-methoxynaphthalene [FL-no: 04.075], is hydroxylated and excreted as a glucuronide.

Concerning their biotransformation, it can be expected that the straight-chain aliphatic ethers included in the subgroup 1 may undergo O-dealkylation *in vivo*, catalysed by P450 to yield the corresponding alcohol and aldehyde that subsequently undergo complete oxidation in the fatty acid pathway and tricarboxylic acid cycle. The demethylated product of 1-methoxy-1-decene [FL-no: 03.022] is an enol, which will rearrange to the aldehyde, which subsequently can be oxidised to the carboxylic acid.

On the basis of information on representative supporting substances, the candidate alicyclic ether 2-acetoxy-1,8-cineole [FL-no: 03.008] and 5-isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyren [FL-no: 13.200] within subgroup 2 may be anticipated to undergo ring-hydroxylation by P450 and conjugation with glucuronic acid followed by excretion in the urine.

The available data suggest that the substance in subgroup 3, alpha-Terpinyll methyl ether [FL no: 03.020] would be metabolised by P450 isoenzymes to yield polar hydroxylated metabolites, which are excreted as glucuronic acid conjugates or further oxidised and then excreted. Cleavage of the ether is a minor metabolic pathway (JECFA, 1999a). The JECFA considered the metabolism of alpha-terpineol at their 51st meeting and they concluded that in humans and animals, terpenoid tertiary alcohols are conjugated primarily with glucuronic acid and are excreted in the urine and faeces. Unsaturated terpenoid alcohols may undergo allylic oxidation to form polar diol metabolites, which may be excreted either free or conjugated. The acetal moiety in vanillin 3-(1-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] is shown to be hydrolysed, resulting in the formation of the corresponding ether and vanillin. It is expected that the alcohol groups in this ether subsequently are oxidised and that the carboxylic acid(s) are conjugated and then excreted or are excreted as the acid itself. Similarly, 1-menthoxy ethanol [FL-no: 02.247] is anticipated to be oxidised to the corresponding carboxylic acid and excreted.

The benzyl ethers found in subgroup 4 [FL-no: 03.011 and 03.012] are expected to be metabolised in a similar way to mono alkyl derivatives of benzene. It is generally accepted that mono-alkyl derivatives of benzene are metabolised by biotransformation of the side chain to produce alcohols and carboxylic acids, which are eliminated in the urine as conjugates of glucuronic acid or glycine (Williams, 1959a).

The candidate aromatic ethers in subgroup 5 [FL-no: 04.059, 04.067, 04.068, 04.069, 04.079, 04.084, 08.127 and 09.687] are expected to be metabolised by ring-hydroxylation (mainly in the para position, cleavage of the methyl ether (O-demethylation) and/or oxidation of the ring substituents depending on the position of substituents. These products would then be expected to be conjugated primarily with glucuronic acid and to a lesser extent sulphate or glycine and excreted in the urine. 2-(4-Methoxyphenoxy)propionic acid [FL-no: 08.127] is expected to be excreted as such or after conjugation.

The naphthol ether [FL-no: 04.075] in subgroup 6 is expected to be excreted as a glucuronic acid conjugate with the methyl ether linkage intact.

Whilst no metabolism data have been found for the candidate substance in subgroup 7, digeranyl ether [FL-no: 03.024], data are available on substances that are supporting for the metabolism of longer chain ethers. Tsuji et al., concluded that dealkylation of ethers becomes less likely as the chain length increases and that ω -oxidation is more likely to occur (Tsuji et al., 1978). The JECFA reviewed the safety of geraniol in 2004 (JECFA, 2004b) and they concluded that ω -oxidation of geraniol was a common metabolic pathway and that this would result in metabolism to innocuous products. EFSA agreed with this view in FGE 72 (EFSA, 2010d). As digeranyl ether contains no free hydroxyl group like geraniol, other pathways for metabolism of geraniol are not available for digeranyl ether and therefore ω -oxidation is more likely to occur. It would be expected that following ω -oxidation, the metabolites would be conjugated with glucuronide and excreted in the urine.

It can be anticipated that the 20 candidate substances [FL-no: 02.247, 02.248, 03.008, 03.011, 03.012, 03.015, 03.016, 03.020, 03.022, 03.024, 04.059, 04.067, 04.068, 04.069, 04.075, 04.079, 04.084, 08.127, 09.687 and 13.200] are metabolised to innocuous products. Although saturation of some metabolic pathways have been described, it occurs at very high doses, unlikely to be reached by the candidate substances when used as flavourings at the estimated levels of intakes.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical groups 15, 16, 22, 26 and 30, and for 20 supporting substances evaluated by the JECFA at the 51st, 59th and 61st meeting (JECFA, 1999a; JECFA, 2003a; JECFA, 2004b). The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Anisole [04.032])	Rat	M, F	Oral	3700	(Taylor et al., 1964)	
	Rat	M, F	Oral	3700	(Bär and Griepentrog, 1967)	
	Rat	M, F	Oral	3700	(Jenner et al., 1964)	
(1-Methoxy-4-methylbenzene [04.015])	Rat	M, F	Oral	1920	(Wong and Hart, 1971)	
(1,3-Dimethyl-4-methoxybenzene [04.063])	Rat	M, F	Oral	> 2000	(Gilman, 1997)	
(1,3-Dimethoxybenzene [04.016])	Rat	NR	Oral	2500	(Moreno, 1978l)	
	Rat	M, F	Oral	2560	(Bär and Griepentrog, 1967)	
(1,4-Dimethoxybenzene [04.034])	Rat	NR	Oral	3600	(Moreno, 1973ae)	
(Prenyl ethyl ether [03.019])	Mouse	M	Oral	24 hours: 1000 – 8000 ; 14 days: 1000 – 4000	(Bähler and Bonetti, 1983)	
Ethyl geranyl ether [03.015]	Rat	NR	Oral	> 5000	(Moreno, 1977ag)	
(1,4-Cineole [03.007])	Rat	NR	Oral	3100	(Moreno, 1981c)	
(1,8-Cineole [03.001])	Rat	M, F	Oral	2480	(Bär and Griepentrog, 1967)	
	Rat	M, F	Oral	2480	(Jenner et al., 1964)	
	Rat	M, F	Oral	1550	(Brownlee, 1940)	
(3,6-Dihydro-4-methyl-2-(2-methylprop-1-en-1-yl)-2H-pyran [13.088])	Rat	NR	Oral	~ 5000	(Moreno, 1980l)	
(2,2,6-Trimethyl-6-vinyltetrahydropyran [13.094])	Rat	M, F	Oral	2700	(Sauer-Freeman, 1980)	
	Mouse	NR	Oral	4000 – 8000	(Roure Bertrand Dupont, 1979)	
(2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran [13.037])	Rat	NR	Oral	4300	(Opdyke, 1976)	
(2-Methoxyethyl benzene [03.006])	Rat	NR	Oral	4100	(Moreno, 1977ah)	
(1-Methoxy-4-propylbenzene [04.039])	Rat	M, F	Oral	4400	(Taylor et al., 1964)	
	Rat	M, F	Oral	4400	(Jenner et al., 1964)	
	Rat	M, F	Oral	4400	(Bär and Griepentrog, 1967)	
	Mouse	NR	Oral	7300	(Jenner et al., 1964)	
(2-Methoxynaphthalene [04.074])	Rat	NR	Oral	> 5000	(Levenstein, 1974h)	
	Mouse	M, F	Oral	825	(Schafer and Bowles, 1985)	
(beta-Naphthyl ethyl ether [04.033])	Rat	M, F	Oral	3110	(Wong and Weir, 1971c)	
	Mouse	M, F	Oral	1213	(Schafer and Bowles, 1985)	
(Isobutyl beta-naphthyl ether [04.054])	Rat	M, F	Oral	5930	(Jenner et al., 1964)	
	Rat	NR	Oral	> 5000	(Moreno, 1978m)	
	Rat	M, F	Oral	5930	(Bär and Griepentrog, 1967)	
(2-Phenoxyethyl isobutyrate [09.487])	Rat	NR	Oral	> 5000	(Moreno, 1973i)	
(Phenoxyacetic acid [08.049])	Rat	M, F	Gavage	1800	(Burdock and Ford, 1990b)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(alpha-Terpineol [02.014])	Rat	M, F	Gavage	1772	(Piccirillo, 1983)	
	Rat	NR	Oral	> 5000	(Moreno, 1976o)	
	Rat	NR	Oral	4300 ¹	(Moreno, 1971)	
	Mouse	M	Gavage	2830	(Yamahara et al., 1985)	
(Terpineol acetate [09.830])	Rat	M, F	Gavage	5075	(Jenner et al., 1964)	

M = Male; F = Female.

NR = Not reported.

¹ Reported for a mixture of alpha- and beta-terpineol.

Subacute / subchronic / chronic / carcinogenicity toxicity data are available for none of the candidate substances of the present Flavouring Group Evaluation from chemical groups 15, 16, 22, 26 and 30 but for 10 supporting substances evaluated by the JECFA at the 51st, 61st meeting (JECFA, 1999a; JECFA, 2004b). The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(1-Methoxy-4-methylbenzene [04.015])	Rat; M, F 20	Gavage	40, 120, 240 mg/kg bw/day	28 days	40	(Brunsberg et al., 1994)	1.
	Rat; M, F 10	Gavage	100, 300, 1000 mg/kg bw/day	28 days (4 weeks)	100	(BASF, 1995)	
(Carvacryl ethyl ether [04.038])	Rat; M, F 10	Diet	22 mg/kg bw/day	14 days	M: < 22 F: 22	(Gill and Van Miller, 1987b)	1.
(1,2-Dimethoxybenzene [04.062])	Rat; M, F 10	Diet	10 mg/kg bw/day	14 days	10	(Trimmer et al., 1992)	1.
(1,3-Dimethoxybenzene [04.016])	Rat; M, F 30	Diet	M: 9.6 mg/kg bw/day F: 11.2 mg/kg bw/day	90 days	M: 9.6 F: 11.2	(Oser et al., 1965)	1.
	Rat; M, F	Diet		84 days (12 weeks)	10	(Bär and Griepentrog, 1967)	1.
	Rat; M, F 20 or 40	Diet	0.1 and 0.5 % equivalent to 50, 250 mg/kg bw/day	730 days (2 years)	250	(Bär and Griepentrog, 1967)	1.
(1,4-Dimethoxybenzene [04.034])	Rat; M, F 5 or 10	Diet	2 % in diet equivalent to 1000 mg/kg bw/day	28 or 56 days	1000	(Altmann et al., 1985)	1.
(1,8-Cineole [03.001])	Rat; M, F 12	Gavage	150, 300, 600, 1200 mg/kg bw/day	28 days	M: 300 F: 1200	(NTP, 1987c)	1.
	Rat; M, F 12	Diet	3750, 7500, 15000, 30000 ppm equal to 381, 766, 1740, 3342 mg/kg bw/day for males and 353, 765, 1527, 3516 mg/kg bw/day for females	28 days	M: Not established F: 1500	(NTP, 1987c)	1.
	Mouse; M, F 12	Gavage	150, 300, 600, 1200 mg/kg bw/day	28 days	1200	(NTP, 1987d)	1. Liver/body weight ratio: 1. vehicle effect males 20 % increase, females 8 % decrease. 2. inconclusive dose-response relationship.
	Mouse; M, F	Diet	3750, 7500,	28 days	M: 562.5	(NTP, 1987d)	1.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	12		15000, 30000 ppm equal to 600, 1322, 2448, 5607 mg/kg bw/day for males and 705, 1532, 3152, 6777 mg/kg bw/day for females		F: 1125		
	Mouse; M 52	Gavage	8, 32 mg/kg bw/day	560 days	32	(Roe et al., 1979)	1. The mice were dosed with a mixture of substances which also included chloroform and peppermint oil. This study is of limited value as only a small number of organs were studied for histopathological changes.
(2-(2-Methylprop-1-enyl)-4-methyltetrahydropyran [13.037])	Rat; M, F 20 - 32	Diet	M: 2.514 mg/kg bw/day F: 2.805 mg/kg bw/day	90 days	M: 2.514 F: 2.805	(Posternak et al., 1969)	1.
(1-Methoxy-4-propylbenzene [04.039])	Rat; M 20	Gavage	Initial dose 2000, gradually increase to 5000 mg/kg bw/day	32 days	Not established	(Hagan et al., 1967)	1.
	Rat; M, F 20	Diet		133 days (19 weeks)	Not established	(Hagan et al., 1967)	1.
(beta-Naphthyl ethyl ether [04.033])	Rat; M, F 30	Diet	M: 5.1 mg/kg bw/day F: 5.7 mg/kg bw/day	90 days	M: 5.1 F: 5.7	(Oser et al., 1965)	1.
	Rat; NR	Diet		84 days (12 weeks)	5.0	(Bär and Griepentrog, 1967)	1.
(Terpineol acetate [09.830])	Rat; M, F 20	Diet	0, 1000, 2500 or 10,000 ppm, equivalent to 0, 50, 125 or 500 mg/kg bw/day	140 days (20 weeks)	500	(Hagan et al., 1967)	2. Very limited details provided.

M = Male; F = Female.

NR = Not reported.

1 Summarised by JECFA 61st meeting (JECFA, 2004b).

2 Summarised by JECFA 51st meeting (JECFA, 1999a).

Developmental and reproductive toxicity data are available for none of candidate substances of the present Flavouring Group Evaluation from chemical groups 15, 16, 22, 26 and 30 but for two supporting substances evaluated by the JECFA at the 59th and 61st meeting (JECFA, 2003a; JECFA, 2004b). Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Species/ Sex No./ group	Route	Dose Levels	Duration	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(1,8-Cineole [03.001])	Rat / F 12 – 17	Gavage	0.16, 0.8, 1.6 ml/kg	Developmental toxicity Gestation days 9 – 14	Maternal: 0.8 ml/kg Foetal: 0.8 ml/kg	(Hasegawa and Toda, 1978)	1 The test substance was Rowachol®, a mixture of α / β -pinene (17 %), l-menthol (32 %), menthone (6 %), borneol (5 %), d-camphene (5 %), 1,8-cineole (eucalyptol) (2 %), and rheochrysin (0.1 %).
(Phenoxyacetic acid [08.049])	Mouse / F 11 / 8 or more	Gavage	Single dose of 800-900 mg/kg bw on on of days 8 - 15 of gestation or three doses of 250 - 300 mg/kg bw at gestation days 7 - 9, 10 - 12 or 13 - 15	Developmental toxicity: One dose (Gestation days 8 - 15) or three doses (Gestation days 7 - 9, 10 - 12 or 13 - 15)	Maternal: NR Foetal: 300 and 900	(Hood et al., 1979)	2.

F = Female.

NR = Not reported.

1 Summarised by JECFA 61st meeting (JECFA, 2004b).

2 Summarised by JECFA 59th meeting (JECFA, 2003a).

In vitro mutagenicity/genotoxicity data are available for three candidate substances of the present Flavouring Group Evaluation from chemical groups 15, 16, 22, 26 and 30 and for 15 supporting substances evaluated by the JECFA at the 51st, 59th and 61st meeting (JECFA, 1999a; JECFA, 2003a; JECFA, 2004b). Supporting substances are listed in brackets.

TABLE IV.4: 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)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	2.
	Sister chromatid exchange	Human lymphocytes	0 - 2 mM	Negative (-S9 only)	(Jansson et al., 1988)	2.
(1-Methoxy-4-methylbenzene [04.015])	Ames reverse mutation assay (plate incorporation method)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	2. Published non-GLP study. Limited report of study details. Validity of the study cannot be evaluated.
	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	50 mg/plate	Negative (+/- S9)	(Heck et al., 1989)	2. 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)	2. 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	<i>S. typhimurium</i> TA100	0.1, 1, 10 and 1000 µg/plate	Negative	(Rapson et al., 1980)	2.
(1,3-Dimethoxybenzene [04.016])	Ames reverse mutation assay	<i>S. typhimurium</i> 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)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	10 - 900 µg/plate	Negative (+/- S9)	(Haworth et al., 1983)	2.
	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	5000 ng/plate	Negative (+/- S9)	(CIT)	.
1,2,3-Trimethoxybenzene [04.084]	Ames reverse mutation assay	<i>S. typhimurium</i> 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	<i>E. coli</i> PQ37	NR	Negative	(Ohshima et al., 1989)	Study assessing the SOS-inducing potency of a range of phenols after nitrosation <i>in vitro</i> in the absence of metabolic activation. The result for 1,2,3-trimethoxybenzene was negative.
(1,8-Cineole [03.001])	Ames reverse mutation assay	<i>S. typhimurium</i> TA97, TA98, TA100, TA102	250 - 2500 µg/plate	Negative (+/- S9)	(Gomes-Carneiro et al., 1998)	2. Published non-GLP study. Fairly detailed

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						description of study details and results, generally follows OECD guidelines. Study considered valid.
	Ames reverse mutation assay (preincubation method)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3.3 - 3333 µg/plate	Negative (+/- S9)	(Haworth et al., 1983)	2. 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.
	Sister chromatid exchange	Chinese hamster ovary cells	50 - 500 µg/ml 600 - 800 µg/ml	Positive (-S9) Negative (+S9)	(Galloway et al., 1987a)	2. 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 K-1 cells	10, 33.3 and 100 µM	Negative (-S9 only)	(Sasaki et al., 1989)	2. 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	479 - 663 µg/ml 630 - 810 µg/ml	Negative (+/- S9)	(Galloway et al., 1987a)	2. 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 hrs.
	Rec assay	<i>B. subtilis</i> H17 (rec+) and M45 (rec-)	18 µg/disk	Negative	(Oda et al., 1979)	2. 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	<i>B. subtilis</i> H17 (rec+) and M45 (rec-)	20 µl/disk (20,000µg/disk)	Negative	(Yoo, 1986)	2. 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	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	750 µg/plate	Negative (+/- S9)	(Wild et al., 1983)	2.
	Unscheduled DNA synthesis	Rat hepatocytes	5 x 10 ⁻³ M	Negative	(Howes et al., 1990)	2.
(2-Methoxynaphthalene [04.074])	Ames reverse mutation assay (plate incorporation method)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	2.
(beta-Naphthyl ethyl ether [04.033])	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate	Negative (+/- S9)	(Wild et al., 1983)	2.

TABLE IV.4: GENOTOXICITY (IN VITRO)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames reverse mutation assay (plate incorporation method)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative (+/- S9)	(Florin et al., 1980)	2.
(Isobutyl beta-naphthyl ether [04.054])	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1 mg/plate	Negative (+/- S9)	(Wild et al., 1983)	2.
(2-Phenoxyethyl isobutyrate [09.487])	Ames reverse mutation assay	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	3600 µg/plate	Negative (+/- S9)	(Wild et al., 1983)	3.
(Phenoxyacetic acid [08.049])	Mutagenicity assay	<i>S. cerevisiae</i> D7tsl	16 mM	Negative (- S9)	(Venkov et al., 2000)	
(Alpha-terpineol [02.014])	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	10000 µg/plate	Negative	(Heck et al., 1989)	4.
	Ames test	<i>S. typhimurium</i> TA97a, TA98, TA100, TA102	2500 µg/plate	Negative ¹	(Gomes-Carneiro et al., 1998)	
	Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate	Negative (+/- S9)	(National Cancer Institute, 1983)	4.
	Spot test	<i>S. typhimurium</i> 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 (467 µg/ml) 0.75 µl/ml (700 µg/ml)	Negative (- S9) Negative (+S9)	(Kirby et al., 1984)	
	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	<i>S. cerevisiae</i>	NR	Negative (+ S9)	(Oda et al., 1979)	4.
(Terpineol acetate [09.830])	Rec assay	<i>B. subtilis</i> H17, M45	19 µg	Negative	(Oda et al., 1979)	4.
Vanillin 3-(1-menthoxy)propane-1,2-diol acetal [02.248]	Ames test	<i>S. typhimurium</i> 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 per concentration.
	Ames test	<i>E. coli</i> 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 per concentration.
5-Isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran [13.200]		<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	Up to 5000 µg/plate	Negative (+/- S9)	(Thompson, 2008)	Valid GLP study.
(Tetrahydro-4-methyl-2-(2-methylpropen-1-yl)pyran [FL-no: 13.037])		<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	Up to 5000 µg/plate	Negative (+/- S9)	(Verspeek-Rip, 2002b)	Valid GLP study.

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).

In vivo mutagenicity/genotoxicity data are available for none of the candidate substances of the present Flavouring Group Evaluation from chemical groups 15, 16, 22, 26 and 30 but for six supporting substances evaluated by the JECFA at the 59th and 61st meeting (JECFA, 2003a; JECFA, 2004b). Supporting substances are listed in brackets.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(1,3-Dimethoxybenzene [04.016])	<i>In vivo</i> Micronucleus test	Mouse	Intraperitoneal injection	558, 966 or 1382 mg/kg bw	Negative	(Wild et al., 1983)	1.
	<i>In vivo</i> Sex- linked recessive lethal mutation assay	<i>D. melanogaster</i>		25 mM	Negative	(Wild et al., 1983)	1.
	<i>In vivo</i> Micronucleus test	Mouse	Oral gavage	2000 mg/kg bw	Negative	(Hoechst, 1996)	
(1,4-Dimethoxybenzene [04.034])	<i>In vivo</i> Micronucleus test	Mouse	Intraperitoneal injection	750, 1125 or 1500 mg/kg	Negative	(Wild et al., 1983)	
(1-Methoxy-4-propylbenzene [04.039])	<i>In vivo</i> Sex- linked recessive lethal mutation assay	<i>D. melanogaster</i>	(751 µg/ml)	5 mM	Negative (+/- S9)	(Wild et al., 1983)	1.
	<i>In vivo</i> Micronucleus test	Mouse	Intraperitoneal injection	1500 mg/kg	Negative	(Wild et al., 1983)	1.
(beta-Naphthyl ethyl ether [04.033])	<i>In vivo</i> Micronucleus test	Mouse	Intraperitoneal injection	344, 603 or 861 mg/kg bw	Negative	(Wild et al., 1983)	1.
	<i>In vivo</i> Sex- linked recessive lethal mutation assay	<i>D. melanogaster</i>		25 mM	Negative	(Wild et al., 1983)	1.
(Isobutyl beta-naphthyl ether [04.054])	<i>In vivo</i> Micronucleus test	Mouse	Intraperitoneal injection	800, 1400 or 2000 mg/kg bw	Negative	(Wild et al., 1983)	1.
	<i>In vivo</i> Sex- linked recessive lethal mutation assay	<i>D. melanogaster</i>		25 mM	Negative	(Wild et al., 1983)	1.
(2-Phenoxyethyl isobutyrate [09.487])	<i>In vivo</i> Micronucleus formation assay	Mouse bone marrow cells	Intraperitoneal injection	1875 mg/kg/bw	Negative	(Wild et al., 1983)	2.
	<i>In vivo</i> Sex-linked recessive mutation	<i>D. melanogaster</i>		10 mM	Negative	(Wild et al., 1983)	

1 Summarised by JECFA 61st meeting (JECFA, 2004b).

2 Summarised by JECFA 59th meeting (JECFA, 2003a).

REFERENCES

- Altmann H-J, Grunow W, Wester PW and Mohr U, 1985. Induction of forestomach lesions by butylhydroxyanisole and structurally related substances. *Arch. Toxicol.* 8, 114-116.
- Asakawa Y, Toyota M and Ishida T, 1988. Biotransformation of 1,4-cineole, a monoterpene ether. *Xenobiotica* 18(10), 1129-1134.
- Asano M and Yamakawa T, 1950. The fate of branched chain fatty acids in the animal body. I. A contribution to the problem of "Hildebrandt acid". *J. Biochem.* 37(3), 321-327.
- Axelrod J, 1956. The enzymic cleavage of aromatic ethers. *Biochem. J.* 63, 634-639.
- Bähler B and Bonetti EP, 1983. Determination of the oral LD50 of prenyl ethyl ether in the mouse. Lab. No. 00260.025. Date between 6/30/83 and 7/13/83. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Bär F and Griepentrog F, 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. [Where we stand concerning the evaluation of flavoring substances from the viewpoint of health]. *Med. Ernähr.* 8, 244-251.
- BASF AG, 1995. BASF AG, department of toxicology, unpublished data, (21C0810/89101), 04-25-1995. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 104-93-8, EINECS Name 4-methylanisole. Section 1.0.1-5.11.
- Bennett, 1997. Vanillin PGA hydrolysis study. Datasheet dated 11/21/1997. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Bernauer U, Amberg A, Scheutzwow D and Dekant W, 1998. Biotransformation of 12C- and 2-13C-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and tert-butyl alcohol in rats: Identification of metabolites in urine by 13C nuclear magnetic resonance and gas chromatography/mass spectrometry. *Chem. Res. Toxicol.* 11, 651-658.
- Brady JF, Xiao F, Ning SM and Yang CS, 1990. Metabolism of methyl tertiary-butyl ether by hepatic microsomes. *Arch. Toxicol.* 64, 157-160.
- Bray HG, James SP, Thorpe WV and Wasdell MR, 1953. The metabolism of ethers in the rabbit. 1. Anisole and diphenyl ether. *Biochem. J.* 54(4), 547-550.
- Bray HG, Craddock VM and Thorpe WV, 1955. Metabolism of ethers in the rabbit. 2. Nuclear-substituted anisoles. *Biochem. J.* 60, 225-232.
- Brownlee G, 1940. A pharmacological examination of cineole and phellandrene. *Q. J. Pharm. Pharmacol.* 13, 130-137.
- Brunsborg B, Meyer O, Würtzen G and Olsen P, 1994. Four-week toxicity study of 4-methoxytoluene in rats. *Toxicol. Lett.* 73, 209-212.
- Burdock GA and Ford RA, 1990b. Acute oral toxicity (LD50) study in the rat with phenoxyacetic acid. *Acute Toxicity Data, J. Am. Coll. Toxicol. Part B*, 1(2), 94-95.
- 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.

- CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.
- Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.
- Daly J and Jerina D, 1969. Migration of deuterium during aryl hydroxylation. Arch. Biochem. Biophys. 134, 266-268.
- Daly J, 1970. Metabolism of acetanilides and anisoles with rat liver microsomes. Biochem. Pharmacol. 19, 2979-2993.
- De-Oliveira ACAX, Fidalgo-Neto AA and Paumgartten FJR, 1999. *In vitro* inhibition of liver monooxygenases by beta-ionone, 1,8-cineole, (-)-menthol and terpineol. Toxicology 135, 33-41.
- Diliberto JJ, Usha G and Birnbaum LS, 1988. Disposition of citral in male Fischer rats. Drug Metab. Disposition 16, 721-727.
- 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, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.
- 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, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
- EFFA, 2003k. Submission 2003-4. Flavouring group evaluation of 12 flavouring substances (candidate chemicals) of the chemical group 15 (Annex I of 1565/2000/EC) structurally related to phenethyl alcohol, aldehyde, acid, and related acetals and esters and related substances [JECFA/WHO FAS 50/59] used as flavouring substances. 9 June 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.22.
- EFFA, 2003l. Submission 2003-4. Flavouring group evaluation of 12 flavouring substances (candidate chemicals) of the chemical group 15 (Annex I of 1565/2000/EC) structurally related to phenethyl alcohol, aldehyde, acid, and related acetals and esters and related substances [JECFA/WHO FAS 50/59] used as flavouring substances. 9 June 2003. FLAVIS/8.22. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat.

- EFFA, 2004af. Submission 2003-6 Addendum. Supplement of three flavouring substances (candidate chemicals) to the flavouring group evaluation of chemical group 22 (Annex I of 1565/2000/EC) structurally related to cinnamyl alcohol and related substances [FAO/WHO JECFA 46/55] used as flavouring substances. June 3, 2004. FLAVIS/4.83. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.
- EFFA, 2004j. Submission 2004-1. Flavouring group evaluation of 16 flavouring substances (candidate chemicals) of the chemical groups 16 and 26 (annex I of 1565/2000/EC) structurally related to aliphatic and aromatic ethers [FEMA 2003-4] used as flavouring substances. 17 March 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.35.
- EFFA, 2004k. Submission 2004-1. Flavouring group evaluation of 16 flavouring substances (candidate chemicals) of the chemical groups 16 and 26 (annex I of 1565/2000/EC) structurally related to aliphatic and aromatic ethers [FEMA 2003-4] used as flavouring substances. 17 March 2004. FLAVIS/8.35. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.
- EFFA, 2010a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
- EFFA, 2011a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
- EFSA, 2004a. Minutes of the 7th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true
- EFSA, 2009a. 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 18, Revision 1: Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical group 6 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 31 January 2008. EFSA-Q-2003-161B.
- EFSA, 2010d. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 72 (FGE.72): Consideration of aliphatic, branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters evaluated by the JECFA (61st meeting) structurally related to branched- and straight-chain unsaturated carboxylic acids. Esters of these and straight-chain aliphatic saturated alcohols evaluated by EFSA in FGE.05Rev2 (2009) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 24 November 2009. EFSA-Q-2009-00561.
- EFSA, 2011k. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 25Rev2: Aliphatic and aromatic hydrocarbons from chemical group 31 (Commission Regulation (EC) No 1565/2000 of 18 July 2000).

- El Masry AM, Smith JN and Williams RT, 1956. Studies in detoxification. 69. The metabolism of alkylbenzenes: n-propylbenzene and n-butylbenzene with further observations on ethylbenzene. *Biochem. J.* 64, 50-56.
- Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.
- Fischer FG and Bielig HJ, 1940. Über die hydrierung ungesättigter stoffe im tierkörper. [On the hydrogenation of unsaturated materials in the animal body]. *Physiol. Chem.* 266, 73-98. (In German)
- Flavour Industry, 2006a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-05.
- Flavour Industry, 2008a. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-23
- Flavour Industry, 2009g. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-23Rev2 [Fl-no: 03.024].
- Flavour Industry, 2010i. Unpublished information submitted by Flavour Industry to the European Food Safety Authority (EFSA) and forwarded to FLAVIS Secretariat. A-23rev3 [Fl-no: 13.200].
- Flavour Industry, 2011b. Unpublished information submitted by Flavour Industry to the European Food Safety Authority (EFSA) and forwarded to FLAVIS Secretariat. Specifications A-23rev3 [FL-no: 02.248].
- 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.
- Gerarde HW and Ahlstrom DB, 1966. The aspiration hazard and toxicity of homologous series of alcohols. *Arch. Environ. Health* 13, 457-461.
- Gill MW and Van Miller JP, 1987b. Fourteen-day dietary minimum toxicity screen (MTS) in albino rats. 3-acetyl-2,5-dimethylthiophene, carvacryl ethyl ether, 2,2'-(thiodimethylene) difuran, 5-methyl-5-hexen-2-one, 5-methyl-2-thiophene carboxaldehyde. Bushy Run Research Center. Project report 50-527. August 31, 1987. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Gilman MR, 1997. Acute oral toxicity study, limit test. 2,4-Dimethylanisole. Celsis Laboratory Group. Assay no. 9717005. November 11, 1997. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- 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.
- Hagan EC, Hansen WH, Fitzhugh OG, Jenner PM, Jones WI, Taylor JM, Long EL, Nelson AA and Brouwer JB, 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. *Food Cosmet. Toxicol.* 5(2), 141-157.

- Hasegawa M and Toda T, 1978. Teratological studies on Rowachol, remedy for cholelithiasis. Effect of Rowachol administered to pregnant rats during organogenesis on pre-and post-natal development of their offspring. *Oyo Yakuri* 15(7), 1109-1119. (In Japanese)
- 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.
- Hiroi T, Miyazaki Y, Kobayashi Y, Imaoka S and Funae Y, 1995. Induction of hepatic P450's in rat by essential wood and leaf oils. *Xenobiotica* 25(5), 457-467.
- Hoechst AG, 1996. Unveröffentl. Unters. (Ber.-Nr. 96.0064 vom 15.02.1996). Cited in European Commission - European Chemicals Bureau, 2000. IUCILID Dataset, Substance ID: 150-78-7, EINECS Name 1,4-dimethoxybenzene. Section 1.0.1-5.11.
- Hood RD, Patterson BL, Thacker GT, Sloan GL and Szczech GM, 1979. Prenatal effects of 2,4,5-T, 2,4,5-trichlorophenol and phenoxyacetic acid in mice. *J. Environ. Sci. Health C13* (3), 189-204.
- Horning MG, Butler CM, Stafford M, Stillwell RN, Hill RM, Zion TE, Harvey DJ and Stillwell WG, 1976. Metabolism of drugs by the epoxide-diol pathway. *Advances in Mass Spectroscopy in Biochemistry and Medicine*, Vol. I. Spectrum Publications, Inc., 91-108.
- 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.
- IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.
- Jäger W, Nasel B, Nasel C, Binder R, Stimpfl T, Vycudilik W and Buchbauer G, 1996. Pharmacokinetic studies of the fragrance compound 1,8-cineol in humans during inhalation. *Chem. Senses* 21(4), 477-480.
- 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, 1981a. 25. Report: Twenty-fifth Meeting of the Joint FAO/WHO Expert Committee on the Food Additives. Report: WHO Technical Report Series, no. 669.
- 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, 1998b. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9-18 June 1998. FAO Food and Nutrition paper 52 Add. 6.
- JECFA, 1999a. Safety evaluation of certain food additives. The fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 42. IPCS, WHO, 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, 2000a. Evaluation of certain food additives. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.
- JECFA, 2002b. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 909. Geneva, 5-14 June 2001.
- JECFA, 2002c. Evaluation of certain food additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 913. Geneva, 4-13 June 2002.
- JECFA, 2002d. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Add. 10.
- JECFA, 2003a. Safety evaluation of certain food additives. Fifty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, 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, 2004a. Evaluation of certain food additives. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 922. Rome, 10-19 June 2003.
- 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, 2005c. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.
- JECFA, 2009c. Evaluation of certain food additives. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 952. Rome, 17-26 June 2008. http://whqlibdoc.who.int/trs/WHO_TRS_952_eng.pdf (May 2009)
- Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh OG, 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2, 327-343.
- Kajiura Y, 1996b. Mutagenicity test of HOTACT 1MM {4-(1-menthoxyethyl)-2-(3'-methoxy-4'-hydroxyphenyl)-1,3-dioxolane}. Central Research Laboratory. Unpublished report submitted by EFSA to FLAVIS Secretariat.

- Kirby PE, Duglas-Tabor Y, Simmons RT, Voglezon RA, Rogers-Back AM, Brauning 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 EFA to FLAVIS Secretariat.
- Kovar KA, Gropper B, Friess D and Ammon HPT, 1987. Blood levels of 1,8-cineole and locomotor activity of mice after inhalation and oral administration of rosemary oil. *Planta Med.* 53(4), 315-318.
- Krantz Jr JC and Carr CJ, 1969. Central nervous system depressants. The general anesthetics. In: *The Pharmacologic Principles of Medical Practice*. 7th Ed. Williams and Wilkins, Baltimore, MD, pp. 89-127.
- Levenstein I, 1974. Acute oral toxicity (rat - 5 gms./kg. body weight dose). Dermal toxicity (rabbit - 5 gms./kg. body weight dose). B-Naphthyl methyl ether. Leberco Laboratories. Assay no. 41780. March 14, 1974. Unpublished data submitted by EFA to FLAVIS secretariat.
- Madyastha KM and Chadha A, 1986. Metabolism of 1,8-cineole in rat: Its effects on liver and lung microsomal cytochrome P-450 systems. *Bull. Environ. Contam. Toxicol.* 37, 759-766.
- Madyastha KM and Srivatsan V, 1988b. Biotransformation of alpha-Terpeniol in the rat: Its effects on the liver microsomal cytochrome P-450 system. *Bull. Environ. Contam. Toxicol.* 41, 17-25.
- Miller MJ, Ferdinandi ES, Klan M, Andrews LS, Douglas JF and Kneiss JJ, 1997. Pharmacokinetics and disposition of methyl tert-butyl ether in Fischer-344 rats. *J. Appl. Toxicol.* 17(Suppl. 1), S3-S12.
- Miyazawa M and Shindo M, 2001. Biotransformation of 1,8-cineole by human liver microsomes. *Nat. Prod. Lett.*, 15(1), 49-53.
- Miyazawa M, Kameoka H, Morinaga K, Negoro K and Mura N, 1989. Hydroxycineole: Four new metabolites of 1,8-cineole in rabbits. *J. Agric. Food Chem.* 37, 222-226.
- Miyazawa M, Shindo M and Shimada T, 2001a. Roles of cytochrome P450 3A enzymes in the 2-hydroxylation of 1,4-cineole, a monoterpene cyclic ether, by rat and human liver microsomes. *Xenobiotica* 31(10), 713-723.
- Miyazawa M, Shindo M and Shimada T, 2001b. Oxidation of 1,8-cineole, the monoterpene cyclic ether originated from *Eucalyptus polybractea*, by cytochrome P4503A enzymes in rat and human liver microsomes. *Drug Metab. Disposition* 29(2), 200-205.
- Moreno OM, 1971. Acute oral LD 50 in rats of dimethyl benzyl carbinyl acetate, pseudo linalyl acetate, oil fir needles siberian, terpineol, alpha-hexyl cinnamate aldehyde. Bio/Dynamics Inc. Project no. 10-71. March 24, 1971. Unpublished data submitted by EFA to FLAVIS Secretariat.
- Moreno OM., 1973ae. Acute oral toxicity in rats. Dermal toxicity in rabbits. Dimethyl hydroquinone. MB Research Laboratories, Inc. Project no. MB 73-234. September 21, 1973. Unpublished data submitted by EFA to FLAVIS Secretariat.
- Moreno OM, 1973i. Acute oral toxicity in rats. Dermal toxicity in rabbits. Phenoxyethyl isobutyrate. MB Research Laboratories, Inc. Project no. MB 73-105. July 5, 1973. Unpublished data submitted by EFA to FLAVIS Secretariat.
- Moreno OM, 1976n. Acute oral toxicity in rats. Dermal toxicity in rabbits. Phenoxy acetic acid. MB Research Laboratories, Inc. Project no. MB 76-1359. October 7, 1976. Unpublished data submitted by EFA to FLAVIS Secretariat.

- Moreno OM, 1976o. Acute oral toxicity in rats. Dermal toxicity in rabbits. Phenoxy acetic acid. MB Research Laboratories, Inc. Project no. MB 76-1359. October 7, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1977ag. Acute oral toxicity in rats. Dermal toxicity in rabbits. Geranyl ethyl ether. MB Research Laboratories, Inc. Project no. MB 77-2016. October 19, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1977ah. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Phenyl ethyl methyl ether. MB Research Laboratories, Inc. Project no. MB 77-392. October 7, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1978l. Acute oral toxicity in rats. Dermal toxicity in rabbits. Dimethyl recorcinol. MB Research Laboratories, Inc. Project no. MB 77-2193. Date 2/08/78. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1978m. Acute oral toxicity in rats. Dermal toxicity in rabbits. beta-Naphthyl isobutyl. MB Research Laboratories, Inc. Project no. MB 77-2210. Date 2/08/78. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1980l. Oral toxicity in rats. Dermal toxicity in rabbits. Nerol oxide. MB Research Laboratories, Inc. Project no. MB 79-4067. Date 2/11/80. Unpublished data submitted by EFFA to FLAVIS Secretariat.
- Moreno OM, 1981c. Oral toxicity in rats. Dermal toxicity in rabbits. Cineole. MB Research Laboratories, Inc. Project no. MB 81-5382. Date 8/31/81. 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.
- NTP, 1987c. Twenty-eight day gavage and encapsulated feed study on 1,8-cineole in fischer 344 rats. NTP Chem. no. 15-NTP study nos. 5014-02 and 5014-06. NCTR study nos. 380 and 439.
- NTP, 1987d. Twenty-eight day gavage and encapsulated feed study on 1,8-cineole in B6C3F1 hybrid mice. NTP Chem. no. 15-NTP study nos. 5014-03 and 5014-07. NCTR study nos. 389 and 440. April 1987.
- 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)
- Ohi H, Takahara E, Ohta S and Hirobe M, 1992. Effects of oxygen concentration on the metabolism of anisole homologues by rat liver microsomes. Xenobiotica 22(11), 1329-1337.
- 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.
- Opdyke DLJ, 1976. Fragrance raw materials monographs: Rose oxide levo. Food Cosmet. Toxicol. 14, 855.
- Oser BL, Carson S and Oser M, 1965. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 3(4), 563-569.
- Parke DV, Rahman KHM and Walker R, 1974a. The absorption, distribution and excretion of linalool in the rat. Biochem. Soc. Trans. 2, 612-615.
- Parke DV, Rahman KHM and Walker R, 1974b. Effect of linalool on hepatic drug-metabolizing enzymes in the rat. Biochem. Soc. Trans. 2, 615-618.

- Pass GJ, McClean S, Stupans I and Davies N, 2001. Microsomal metabolism of the terpene 1,8-cineole in the common brushtail possum (*Trichosurus vulpecula*), koala (*Phascolarctos cinereus*), rat and human. *Xenobiotica* 31(4), 205-221.
- Phillips JC, Kingsnorth J, Gangolli SD and Gaunt II, 1976. Studies on the absorption, distribution and excretion of citral in the rat and mouse. *Food. Cosmet. Toxicol.* 14, 537-540.
- Piccirillo VJ, 1983. Acute oral toxicity (LD50) study in the rat with phenoxyacetic acid. Borriston Laboratories, Inc. Borriston Project no. 2901(5). January 31, 1983. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Posternak NM, Linder A and Vodoz CA, 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. *Food Cosmet. Toxicol.* 7, 405-407.
- Rapson WH, Nazar MA and Butzky VV, 1980. Mutagenicity produced by aqueous chlorination of organic compounds. *Bull. Environ. Contam. Toxicol.* 24, 590-596.
- Reitz G, 1995. Hydrolysis of Vanillin TK-10 acetal (TAK#935011). Report with cover letter dated 01/12/95. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Roe FJ, Palmer AK, Worden AN and Van Abbé NJ, 1979. Safety evaluation of toothpaste containing chloroform. I. Long-term studies in mice. *J. Environ. Pathol. Toxicol.* 2, 799-819.
- Roure Bertrand Dupont, 1979. Orientierende prüfung auf acute intraperitoneale und orale toxischkeit an der SPF-albino maus. Trimethyl 2,2,6-vinyl-6-tetrahydropyran. June 1979. Unpublished report submitted by EFFA to FLAVIS Secretariat. (In German)
- Sangster SA, Caldwell J, Hutt AJ and Smith RL, 1983. The metabolism of p-propylanisole in the rat and mouse and its variation with dose. *Food Chem. Toxicol.* 21(3), 263-271.
- Sangster SA, Caldwell J, Hutt AJ and Smith R, 1987. The metabolic disposition of [methoxy-14C]-labelled trans-anethole, estragole and p-propylanisole in human volunteers. *Xenobiotica* 17(10), 1223-1232.
- 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.
- Sauer-Freeman C, 1980. Acute oral median lethality toxicity in rats (LD50) with compound 80-011-01. Cosmopolitan Safety Evaluation, Inc. 14 August, 1980. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.
- 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.
- Schafer EW and Bowles WA, 1985. The acute oral toxicity and repellency of 933 chemicals to house and deer mice. *Arch. Environ. Contam. Toxicol.* 14, 111-129.
- Takahara E, Ohta S and Hirobe M, 1986. Effect of oxygen concentration on the metabolic pathway of anisole in rat liver. *Biochem. Pharmacol.* 35(3), 541-544.

- Taylor JM, Jenner PM and Jones WI, 1964. A comparison of the toxicity of some allyl, propenyl and propyl compounds in the rat. *Toxicol. Appl. Pharmacol.* 6, 378-387.
- Thompson PW, 2008. Reverse mutation assay "Ames test" using *Salmonella typhimurium* (5- isopropyl-2,6-diethyl-2-methyltetrahydro-2H-pyran). Harlan Laboratories Ltd, UK. Project nr. 2082/0175. 3 December 2008. Unpublished report submitted by Industry to FLAVIS Secretariat.
- TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- TNO, 2011. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.
- Trimmer GW, Phillips RD and Damico JS, 1992. 14-Day subchronic oral toxicity study in the rat. 1,2-Dimethoxybenzene. Exxon Biomedical Sciences, Inc. Project no. 129370. August 13, 1992. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Tsuji H, Yoshimura H and Tsukamoto H, 1978. Metabolism of butyl p-nitrophenyl ether *in vitro* with rabbit liver preparations. *Xenobiotica*, 8(4), 245-251.
- 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.
- Ventura P, Schiavi M and Serafini S, 1985. Further studies of trans-sobrerol metabolism: rat, dog and human urine. *Xenobiotica* 15(4), 317-325.
- Verspeek-Rip CM, 2002b. Evaluation of the mutagenic activity of rose oxide co in the *Salmonella typhimurium* reverse mutation assay. NOTOX Project 359448. October 30, 2002. Unpublished report submitted by Industry to FLAVIS Secretariat.
- 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.
- Williams RT, 1959a. Detoxication mechanisms. The metabolism and Detoxification of Drugs, Toxic Substances, and Other Organic Compounds. 2nd Ed. Chapman & Hall Ltd, London.
- Wong LCK and Hart ER, 1971. Acute oral toxicity studies - rats. Acute dermal toxicity studies - rabbits. Primary skin irritation - rabbits. 17 fragrance materials. Final report. Bionetics Research Laboratories. July 30, 1971. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Wong LCK and Weir RJ, 1971c. Acute oral toxicity studies - rats. Acute dermal toxicity studies - rabbits. Primary skin irritation - rabbits. Anise oil, olibanum, anisyl acetate, phenyl propyl alcohol, amyl benzoate, nerolin. Bionetics Research Laboratories. August 25, 1971. Unpublished report submitted by EFFA to FLAVIS Secretariat.
- Yamahara J, Kimura H, Kobayashi M, Okamoto T, Sawada T, Fujimura H and Chisaka T, 1985. Cholagogic action and characteristics of (+/-) alpha-terpineol-beta-D-O-glucopyranoside, a new monoterpenoid glucoside. *Chem. Pharm. Bull.* 33(4), 1669-1675.
- Yoo YS, 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. *Osaka City Med. J.* 34(3-4), 267-288.

ABBREVIATIONS

ADI	Acceptable Daily Intake
AUC	Area Under Curve
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EROD	EthoxyResorufin-O-Deethylase
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)
GI	Gastrointestinal
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	Lethal Dose, 50%; Median lethal dose
MC	MethylCholanthrene
MROD	MethoxyResorufin-O-Demethylase
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
MTBE	Methyl Tertiary-Butyl Ether
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level

NTP	National Toxicology Program
PB	PhenoBarbital
PROD	PentoxylResorufin-O-Depenylase
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
MTBE	Methyl Tertiary-Butyl Ether
TAMDI	Theoretical Added Maximum Daily Intake
TBA	Tertiary-Butyl Alcohol
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation