EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic hydrocarbons evaluated by EFSA in FGE.25Rev3

Nørby, Karin Kristiane; Beltoft, Vibe Meister; EFSA publication

Link to article, DOI:
10.2903/j.efsa.2015.4067

Publication date:
2015

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

European Food Safety Authority

EFSA Journal 2015;13(4):4067

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic hydrocarbons evaluated by EFSA in FGE.25Rev3[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns 21 aromatic, aliphatic and alicyclic hydrocarbons [FL-no: 01.002, 01.003, 01.004, 01.005, 01.006, 01.007, 01.008, 01.009, 01.010, 01.016, 01.017, 01.018, 01.019, 01.020, 01.024, 01.026, 01.029, 01.040, 01.045, 01.061 and 01.077] evaluated by the JECFA in 2005. FGE.78Rev1 considered 24 substances, but 4-methyl-1,1'-biphenyl (JECFA-no: 1334) and biphenyl (JECFA-no: 1332) [former FL-no: 01.011 and 01.013] are no longer supported by Industry for use as a flavouring substance in Europe. Moreover 1-methylnaphthalene [FL-no: 01.014] is in the process of being deleted from the Union List. This revision is owing to additional genotoxicity data on β-caryophyllene [FL-no: 01.007] and 90-day studies in rats on β-caryophyllene [FL-no: 01.007] and myrcene [FL-no: 01.008]. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The specifications for the materials of commerce are adequate for all substances. The Panel concluded that the 21 aromatic, aliphatic and alicyclic hydrocarbons [FL-no: 01.002-01.010, 01.016-01.020, 01.024, 01.026, 01.029, 01.040, 01.045, 01.061 and 01.077] do not give rise to safety concern at their levels of dietary intake, estimated on the basis of the MSDI approach.

[1] On request from the European Commission, Question No EFSA-Q-2013-00185 to -00192 and EFSA-Q-2013-00845 to -00848, adopted on 18 March 2015.

[2] Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Konrad Grob, Rainer Gürtler, Trine Husøy, Wim Mennes, Maria Rosaria Milana, André Penninks, Maria de Fatima Tavares Poças, Vittorio Silano, Andrew Smith, Christina Tlustos, Fidel Toldra, Detlef Wölfle and Holger Zorn. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby and EFSA staff: Annamaria Rossi, Maria Carfi and Maria Anastassiadou for the support provided to this scientific opinion.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific Opinion on Flavouring Group Evaluation 78, Revision 2 (FGE.78Rev2): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic hydrocarbons evaluated by EFSA in FGE.25Rev3. EFSA Journal 2015;13(4):4067, 72 pp. doi:10.2903/j.efsa.2015.4067

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

KEY WORDS

flavouring, aliphatic, alicyclic, aromatic, hydrocarbons, JECFA, 63rd meeting, FGE.78Rev2, FGE.25Rev3.

SUMMARY

Following a request from the European Commission the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the CEF Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The present revision of FGE.78, FGE.78Rev2, includes the assessment of additional available toxicity data for β-caryophyllene [FL-no: 01.007] and myrcene [FL-no: 01.008]. The data provided are for both substances a new 90-day study. Furthermore, new short term study and genotoxicity data have been provided for [FL-no: 01.007]. In addition, since the publication of FGE.78Rev1, two ([FL-no: 01.011 and 01.013]) of the 24 substances are no longer supported for use as flavouring substances in Europe by Industry. Moreover, [FL-no: 01.014] is in the process of being deleted from the Union List. Accordingly, FGE.78Rev2 only deals with 21 substances.

The Panel concluded that the 21 substances in the JECFA flavouring groups of aliphatic and alicyclic and aromatic hydrocarbons are structurally related to the group of 14 aliphatic hydrocarbons evaluated by EFSA in the Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3).

The Panel agrees with the application of the Procedure as performed by the JECFA for eight of the 21 substances considered in this FGE [FL-no: 01.002, 01.005, 01.006, 01.016, 01.019, 01.020, 01.045 and 01.077].

For the following 13 substances [FL-no: 01.003, 01.004, 01.007, 01.008, 01.009, 01.010, 01.017, 01.018, 01.024, 01.026, 01.029, 01.040 and 01.061] it cannot be concluded that they are metabolised to innocuous substances and therefore their evaluation should proceed along the B-side of the Procedure. For one of these substances, 1-isopropenyl-4-methylbenzene [FL-no: 01.010] from subgroup IVe (alkyl substituted benzene hydrocarbons), a NOAEL was available giving an adequate margin of safety compared to the estimated level of intake.

For eight substances [FL-no: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026 and 01.029] adequate margins of safety compared to the estimated levels of intake were estimated based upon a NOAEL from a 90-day study in rats, for the representative substance ß-caryophyllene [FL-no: 01.007]. The NOAEL of 222 mg/kg bw per day provides a margin of safety of 40 000 for ß-caryophyllene. The margins of safety for [FL-no: 01.003, 01.004, 01.009, 01.017, 01.024, 01.026 and 01.029] based upon the respective MSDI for these substances and the NOAEL for ß-caryophyllene, range between 7400 and 1.1×10^7. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be evaluated at step B4 in the Procedure as being of no safety concern.

For four substances [FL-no: 01.008, 01.018, 01.040 and 01.061] adequate margins of safety compared to the estimated levels of intake were estimated based upon a NOAEL from a 90-day study in rats, for the representative substance myrcene [FL-no: 01.008]. The NOAEL of 44 mg/kg bw per day provides a margin of safety of 9100 for myrcene. The margins of safety for [FL-no 01.018, 01.040 and 01.061] based upon the respective MSDI for these substances and the NOAEL for myrcene are 4.8×10^5, 4.4×10^6 and 1.1×10^7, respectively. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be evaluated at step B4 in the Procedure as being of no safety concern.

For four substances use levels have been provided by the Industry [FL-no: 01.008, 01.011, 01.013 and 01.026] and the mTAMDI have been considered. The mTAMDI figures calculated were above the threshold of concern for all four substances and more reliable exposure data are needed. On the basis of such additional data these flavouring substances should be reconsidered using the Procedure. For the remaining 17 substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment to finalise the evaluation.

In order to determine whether the conclusion for the 21 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications.

Adequate specifications including complete purity criteria and identity are available for all 21 JECFA evaluated substances.

Thus, for the 21 substances[4] [FL-no: 01.002, 01.003, 01.004, 01.005, 01.006, 01.007, 01.008, 01.009, 01.010, 01.016, 01.017, 01.018, 01.019, 01.020, 01.024, 01.026, 01.029, 01.040, 01.045, 01.061 and 01.077] the Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

[4] The 22nd substance, 1-methylnaphthalene [FL-no: 01.014] is in the process of being deleted from the Union List (DG SANTE, 2015).

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[5] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[6]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[7].

FGE.78Rev1

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2[8].

The substances [FL-no: 01.008, 01.018, 01.040 and 01.061] were among the 14 substances for which the Panel had "reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing information on stereoisomerism/composition of mixture)" and also among those for which "additional toxicity data was requested".

FGE.25Rev2

On 19 May 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31[9].

The substances with [FL-no: 01.035, 01.064, 01.070 and 01.035] were among the 27 candidate substances for which "additional toxicity data" were required by EFSA. For [FL-no: 01.035] also "additional information on composition" was requested.

FGE.18Rev2

On 30 September 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 18, Revision 2 (FGE.18Rev2): Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters from chemical groups 6 and 8.

For the flavouring substance [FL-no: 02.146], the Panel considered that "additional data" are needed including "information on specifications/stereoisomerism/composition of mixture".

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

[6] Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1-161.

[7] 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. OJ L 180, 19.7.2000, p. 8-16.

[8] EFSA Journal 2011;9(6):2178

[9] EFSA Journal 2011;9(6):2177

On 21 November 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted a statement on the re-evaluation of 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] based on additional data on a supporting substance[10].

The Panel concluded that "linalool [FL-no: 02.013] is not sufficiently structurally related to 3,7-dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] for a re-evaluation of [FL-no: 02.146]". Accordingly, "a 90-day study on 3,7- dimethylocta-1,5,7-trien-3-ol [FL-no: 02.146] or on a sufficiently structurally related substance has to be provided in order to establish on appropriate NOAEL".

New data and relationship with other substances

On 5 and 11 July 2013, the applicant submitted additional data on the following acyclic terpene hydrocarbons [FL-no: 01.008, 01.018, 01.040, 01.061, 01.035, 01.064, 01.070 and 02.146, represented by myrcene [FL-no: 01.008].

As regards the related substances also evaluated in these opinions, namely [FL-no: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026, 01.029 and 01.059], data was submitted and are currently being evaluated (EFSA-Q-2013-00185 to – 00193).

As regards substance with [FL-no: 01.014], data should be submitted by 31 December 2013[11].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment on this group of flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

SUPPORTING DOCUMENTS

Submission by the European Flavour Association

INTERPRETATION OF THE TERMS OF REFERENCE

The above background and terms of reference include also a previous mandate received from the European Commission on 6 February 2013[12]. The present scientific opinion FGE.78Rev2 covers the safety assessment of the following flavouring substances: Pin-2(10)-ene with [FL-no: 01.003], Pin-2(3)-ene with [FL-no: 01.004], beta-caryophyllene with [FL-no: 01.007], Camphene with [FL-no: 01.009], Valencene with [FL-no: 01.017], beta-Bourbonene with [FL-no: 01.024], 1(5),7(11)-Guaiadiene with [FL-no: 01.026], delta-3-Carene [FL-no: 01.029], Myrcene with [FL-no: 01.008], beta-Ocimene with [FL-no: 01.018], alpha-Farnesene with [FL-no: 01.040] and Undeca-1,3,5-triene with [FL-no: 01.061].

[10] EFSA Journal 2012;10(12):2995
[11] This substance is in the process of being deleted from the Union List (DG SANTE, 2015)
[12] SANCO.E3/SH/km D (2013) Ares(2013)15188

ASSESSMENT

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

The following issues are of special importance.

Intake

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

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

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

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

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

Threshold of 1.5 Microgram/Person per day (Step B5) Used by the JECFA

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

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional

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

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

Genotoxicity

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

Specifications

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

Structural Relationship

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

1. History of the Evaluation of the Substances in the Present FGE

At its 63rd meeting the JECFA evaluated a group of 20 flavouring substances consisting of aliphatic and alicyclic hydrocarbons and another group of five aromatic hydrocarbons (JECFA, 2006a). One of the substances, *d*-limonene ([FL-no: 01.045], JECFA-no: 1326), was at the 39th meeting allocated an acceptable daily intake (ADI) of 0 - 1.5 mg/kg body weight (bw). This ADI was withdrawn at the 41st meeting and replaced by an "ADI not specified". One of the substances in the group of aliphatic and alicyclic hydrocarbons is not in the Register (cadinene – mixture of isomers, JECFA-no: 1346). FGE.78 therefore dealt with 24 JECFA evaluated aliphatic, alicyclic and aromatic hydrocarbons (EFSA, 2009).

The Revision 1 of FGE.78, FGE.78Rev1, concerned the re-consideration of one JECFA evaluated substance considered in FGE.78. In FGE.78, the Panel concluded that for 13 substances no applicable NOAEL was available for the substance itself or on a structurally related compound and therefore further data were required. Additional data (long term study of toxicity, mutagenicity studies and new tonnage figure) became available for myrcene [FL-no: 01.008] and the FGE.78Rev1 included the evaluation of these data submitted by the Industry (Flavour Industry, 2010). Furthermore, after publication of FGE.78, the JECFA re-evaluated flavouring substances for which new tonnage data were submitted to the JECFA by Industry. These tonnage figures were included in FGE.78Rev1 for [FL-no: 01.029 and 01.040] (EFSA CEF Panel, 2011a).

Since the publication of FGE.78Rev1, [FL-no: 01.011, 01.013 and 01.014] are no longer supported for use as flavouring substances in Europe by Industry (DG SANCO, 2012 and DG SANTE, 2015) and will therefore not be considered any further in the present FGE. For this, the three listed below

substances will be excluded from this revision except in tables 3 and 11. Information in the text on these substances will be kept only if relevant for the remaining substances.

FL-no JECFA-no	EU Register name
01.011 1334	4-Methyl-1,1'-biphenyl
01.013 1332	Biphenyl
01.014	1-Methyl-naphthalene

Accordingly, FGE.78Rev2 deals with 21 substances.

The table below gives information on publication dates and links to the published versions.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
78	6 March 2008	http://www.efsa.europa.eu/en/scdocs/scdoc/931.htm	24
78Rev1	18 May 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2178.htm	24
78Rev2	18 March 2015		21

The present revision of FGE.78, FGE.78Rev2, includes additional toxicity data provided for two representative substances β-caryophyllene [FL-no: 01.007] and myrcene [FL-no: 01.008]. β-Caryophyllene [FL-no: 01.007] has been selected as representative substance for pin-2(10)-ene [FL-no: 01.003], pin-2(3)-ene [FL-no: 01.004], camphene [FL-no: 01.009], valencene [FL-no: 01.017], β-bourbonene [FL-no: 01.024], 1(5),7(11)-guaiadiene [FL-no: 01.026] and δ-3-carene [FL-no: 01.029]. Myrcene [FL-no: 01.008] has been selected as representative substance for β-ocimene [FL-no: 01.018], α-farnesene [FL-no: 01.040] and undeca-1,3,5-triene [FL-no: 01.061] (EFSA CEF Panel, 2012). The data provided are for each of the two representatives a 90-day study. Furthermore, a new short term study and genotoxicity data have been provided for [FL-no: 01.007] (EFFA, 2012). A search in open literature was conducted for genotoxicity for the two representative substances β-caryophyllene [FL-no: 01.007] and myrcene [FL-no: 01.008]. This search did not reveal any pertinent new information on the substances.

Information on isomerism or on composition of mixture had been provided for 14 substances [FL-no: 01.004, 01.007, 01.008, 01.009, 01.017, 01.018, 01.019, 01.020, 01.024, 01.026, 01.029, 01.040, 01.045 and 01.061] (EFFA, 2013; EFFA, 2015) and EU production volume has been provided for one substance [FL-no: 01.024] (EFFA, 2012) since the previous evaluation of FGE.78.

2. Presentation of the Substances in the JECFA Flavouring Group

2.1. Description

2.1.1. JECFA Status

The JECFA has evaluated two groups of flavouring substances at their 63rd meeting:

- a group of 20 substances consisting of aliphatic and alicyclic hydrocarbons
- a group of five aromatic hydrocarbons

One of the substances, *d*-limonene ([FL-no: 01.045], JECFA-no: 1326), was at the 39th meeting allocated an acceptable daily intake (ADI) of 0-1.5 mg/kg body weight (bw). This ADI was withdrawn at the 41st meeting and replaced by an "ADI not specified".

One of the substances in the group of aliphatic and alicyclic hydrocarbons is not in the Register (cadinene – mixture of isomers, JECFA-no: 1346). Three substances, 4-methyl-1,1'-biphenyl (JECFA-no: 1334), biphenyl (JECFA-no: 1332) and 1-methylnaphthalene [former FL-no: 01.011, 01.013 and 01.014] are no longer supported for use as flavouring substances in Europe by Industry. This consideration will therefore deal with 21 JECFA evaluated aliphatic, alicyclic and aromatic hydrocarbons.

2.1.2. EFSA Considerations

The Panel concluded that the 21 substances in the JECFA flavouring group of aliphatic, alicyclic and aromatic hydrocarbons are structurally related to the group of aliphatic hydrocarbons evaluated by EFSA in the Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3).
Because of the structural diversity within this group, EFSA subdivided the substances considered in FGE.25Rev3 into subgroups. For the sake of clarity and consistency, the substances evaluated previously by the JECFA which are considered in the present evaluation have been allocated to the corresponding subgroups. The subgrouping is shown in Table 4 in Section 6.3.

2.2. Isomers

2.2.1. Status

The following 10 substances [FL-no: 01.003, 01.004, 01.006, 01.007, 01.009, 01.017, 01.024, 01.026, 01.029 and 01.045] in the group of 21 JECFA evaluated aliphatic, alicyclic and aromatic hydrocarbons have one or more chiral centres and the following three [FL-no: 01.018, 01.040 and 01.061] can exist as geometrical and/or other isomers.

2.2.2. EFSA Considerations

Adequate information on isomerism and composition data has been provided for all these substances.

2.3. Specifications

2.3.1. Status

The JECFA specifications are available for all 21 substances (JECFA, 2005a) (see Table 3).

2.3.2. EFSA Considerations

The available specifications are considered adequate for all 21 substances (see Section 2.2.1).

3. Intake Estimation

3.1. Status

For all substances evaluated through the JECFA Procedure, EU production volumes are available (see Table 11).

3.2. EFSA Considerations

Only for two of the 21 JECFA evaluated substances normal and maximum use levels have been provided by the Flavour Industry (EFFA, 2005) (see Table 2). Based on these normal use levels, the intakes based on mTAMDI (see Table 1) can be calculated (EC, 2000; EFSA, 2004).

Table 1: Estimated Intakes Based on the MSDI- and the mTAMDI Approach – FGE.78Rev2

FL-no	EU Register name	MSDI – EU (µg/*capita* per day)	MSDI – USA (µg/*capita* per day)	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per day)
01.002	1-Isopropyl-4-methylbenzene	926	472		Class I	1800
01.003	Pin-2(10)-ene	1300	759		Class I	1800
01.005	Terpinolene	660	70		Class I	1800
01.006	alpha-Phellandrene	79	410		Class I	1800
01.007	beta-Caryophyllene	330	508		Class I	1800
01.009	Camphene	13	28		Class I	1800
01.010	1-Isopropenyl-4-methylbenzene	18	0.3		Class I	1800
01.016	1,4(8),12-Bisabolatriene	13	10		Class I	1800
01.017	Valencene	53	26		Class I	1800
01.018	beta-Ocimene	55	11		Class I	1800
01.019	alpha-Terpinene	28	93		Class I	1800
01.020	gamma-Terpinene	1200	321		Class I	1800
01.024	beta-Bourbonene	0.012	0.2		Class I	1800
01.026	1(5),7(11)-Guaiadiene	0.012	3	3900	Class I	1800
01.029	delta-3-Carene	290	40		Class I	1800
01.040	alpha-Farnesene	0.61	40		Class I	1800
01.061	Undeca-1,3,5-triene	0.24	0.2		Class I	1800
01.077	1-Methyl-1,3-cyclohexadiene	0.012	313		Class I	1800
01.004	Pin-2(3)-ene	1800	2444		Class I	1800
01.008	Myrcene	290	153	3900	Class I	1800
01.045	d-Limonene	34000	12726		Class I	1800

Table 2: Normal and Maximum Use Levels (mg/kg) Available for JECFA evaluated Substances in FGE.78Rev2

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**
01.008	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
01.026	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

SUMMARY OF SPECIFICATION DATA

Table 3: Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2005a)

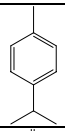
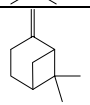
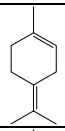
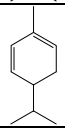
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
01.002 1325	1-Isopropyl-4-methylbenzene		2356 620 99-87-6	Liquid $C_{10}H_{14}$ 134.21	Insoluble Soluble	177 IR 97 %	1.484-1.491 0.853-0.855	
01.003 1330	Pin-2(10)-ene		2903 2114 127-91-3	Liquid $C_{10}H_{16}$ 136.24	Insoluble Insoluble	163-166 NMR 97 %	1.476-1.482 0.867-0.871	Racemate.
01.004 1329	Pin-2(3)-ene		2902 2113 80-56-8	Liquid $C_{10}H_{16}$ 136.24	Insoluble Soluble	155 NMR 97 %	1.462-1.468 0.855-0.860	Racemate. According to the JECFA: ”Min. assay value may include traces of limonene, ß-pinene and other common $C_{10}H_{16}$ terpenes”. The traces are 1-3 % limonene (racemate), 1-3 % β-pinene (racemate) and 1-3 % other ”common terpenes” (α-terpinene, β-terpinene, δ-3-carene, terpinolene etc (each < 1 %)) (EFFA, 2015).
01.005 1331	Terpinolene		3046 2115 586-62-9	Liquid $C_{10}H_{16}$ 136.24	Insoluble Insoluble	183-185 NMR 95 %	1.474-1.484 0.872-0.882	
01.006 1328	alpha-Phellandrene		2856 2117 99-83-2	Liquid $C_{10}H_{16}$ 136.24	Insoluble Soluble	175 IR 95 %	1.471-1.477 0.845-0.855	Racemate.

Table 3: Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2005a)

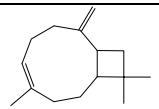
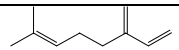
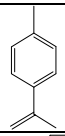
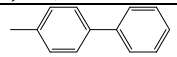
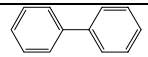
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
01.007 1324	beta-Caryophyllene		2252 2118 87-44-5	Liquid $C_{15}H_{24}$ 204.36	Insoluble Soluble	256 IR 80 %	1.498-1.504 0.899-0.908	80-92 % β-caryophyllene; 10-15 % α-humulene; 5-6 % valencene and other $C_{15}H_{24}$ hydrocarbons as isolated from natural sources.
01.008 1327	Myrcene		2762 2197 123-35-3	Liquid $C_{10}H_{16}$ 136.24	Insoluble Soluble	166-167 NMR 90 %	1.466-1.471 0.789-1.793	At least 90 % myrcene; secondary components 1-5 % limonene, 1-2 % ocimene and trace amounts of pinene and dimers of myrcene.
01.009 1323	Camphene		2229 2227 79-92-5	Solid $C_{10}H_{16}$ 136.24	Insoluble Soluble	n.a. 52 NMR 80 %	n.a. n.a.	Racemate. At least 80 % camphene; secondary components 10-12 % Fenchene; 4-5 % limonene and trace amounts of pinene, myrcene and other terpene hydrocarbons.
01.010 1333	1-Isopropenyl-4-methylbenzene		3144 2260 1195-32-0	Liquid $C_{10}H_{12}$ 132.20	Insoluble Soluble	186-189 NMR 97 %	1.532-1.535 0.846-0.854	
01.011 1334	4-Methyl-1,1'-biphenyl		3186 2292 644-08-6	Solid $C_{13}H_{12}$ 168.24	Insoluble Soluble	n.a. 49-50 NMR 98 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
01.013 1332	Biphenyl		3129 10978	Solid $C_{12}H_{10}$	Insoluble Soluble	254 69	n.a. n.a.	No longer supported by Industry (DG SANCO,

Table 3: Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2005a)

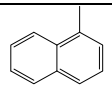
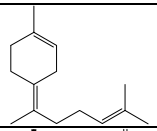
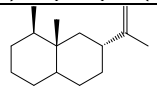
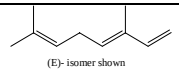
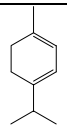
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
			92-52-4	154.21		NMR 99 %		2012).
01.014 1335	1-Methylnaphthalene		3193 11009 90-12-0	Liquid $C_{11}H_{10}$ 142.20	Insoluble Soluble	241-245 NMR 97 %	1.612-1.618 1.020-1.025	No longer supported by industry (DG SANTE, 2015).
01.016 1336	1,4(8),12-Bisabolatriene		3331 10979 495-62-5	Liquid $C_{15}H_{24}$ 204.36	Insoluble Insoluble	262 NMR 97 %	1.493-1.497 0.850-0.858	
01.017 1337	Valencene		3443 11030 4630-07-3	Liquid $C_{15}H_{24}$ 204.36	Insoluble Insoluble	123 (14 hPa) NMR 94 %	1.498-1.508 0.914-0.919	CASrn refers to (+)-Valencene. At least 94 % valencene; secondary components 1-2 % bisabolene and 1-2 % farnesene.
01.018 1338	beta-Ocimene	 (E)- isomer shown	3539 11015 13877-91-3	Liquid $C_{10}H_{16}$ 136.24	Insoluble Soluble	177 NMR 80 %	1.478-1.491 0.801-0.805	80 %; mixture of (E)- and (Z)-isomers. 50-70 % (E)-isomer (EFFA, 2014). Secondary component 15-17 % cis-ß-Ocimene. CASrn in Register does not specify stereoisomeric composition.
01.019 1339	alpha-Terpinene		3558 11023 99-86-5	Liquid $C_{10}H_{16}$ 136.24	Insoluble Soluble	173 NMR 89 %	1.475-1.480 0.833-0.838	At least 89 % α-terpinene; secondary components 3-5 % 1,8- and 2-3 % 1,4-cineole (EFFA, 2014).

Table 3: Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2005a)

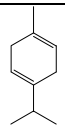
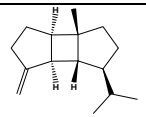
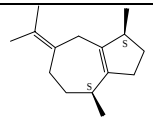
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
01.020 1340	gamma-Terpinene		3559 11025 99-85-4	Liquid $C_{10}H_{16}$ 136.24	Insoluble Soluble	182 NMR 95 %	1.472-1.478 0.841-0.845	According to JECFA: Min. assay value is "95 %" and "may include traces of limonene, alpha and beta- pinene and other common $C_{10}H_{16}$ terpenes".
01.024 1345	beta-Bourbonene		11931 5208-59-3	Liquid $C_{15}H_{24}$ 204.36	Insoluble Soluble	121 (14 hPa) NMR 96 %	1.500-1.507 0.899-0.908	Stereoisomeric composition specified by CASrn and name in Register. According to JECFA: Min. assay value is ”96 %” and ”may include traces of other $C_{15}H_{24}$ compounds (cadinene, guaiene, farnesene)”. 4 % consist of $C_{15}H_{24}$ compounds.
01.026 1347	1(5),7(11)-Guaiadiene		88-84-6	Liquid $C_{15}H_{24}$ 204.36	Insoluble Soluble	118 (3 hPa) NMR 96 %	1.503-1.509 0.912-0.918	Stereoisomeric composition specified by CASrn and name in Register. According to the JECFA: Min. assay value is ”96 %” and ”may include traces of other $C_{15}H_{24}$ compounds (cadinene, farnesene, valencene)”. 4 % consist of $C_{15}H_{24}$ compounds.

Table 3: Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2005a)

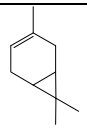
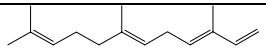
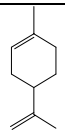
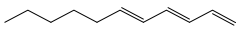
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
01.029 1342	delta-3-Carene		3821 10983 13466-78-9	Liquid $C_{10}H_{16}$ 136.24	Insoluble Slightly soluble	169-174 NMR 92 %	1.468-1.478 0.860-0.868	Racemate. At least 92 %; secondary components 2-3 % β-pinene; 1-2 % limonene; 1-2 % myrcene; 0-1 % p-cymene.
01.040 1343	alpha-Farnesene		3839 10998 502-61-4	Liquid $C_{15}H_{24}$ 204.36	Insoluble Slightly soluble	53-57 (1 hPa) NMR 67 %	1.490-1.500 0.834-0.845	38-50 % α-isomer, 29-40 % ß-isomer and secondary components: 10-15 % bisabolene and up to 2-3 % each of valencene, bourbonene, cadinene and guaiene (EFFA, 2014).
01.045 1326	d-Limonene		2633 491 5989-27-5	Liquid $C_{10}H_{16}$ 136.24	Insoluble Soluble	175-177 IR 96 %	1.471-1.477 0.838-0.843	According to the JECFA: Min. assay value is ”96 % (sum of d/l isomers)” and ”Compounds present above 0.5 %: linalool, myrcene”. In commerce d-Limonene is defined as 90-93 % d-isomer and 2-6 % l-isomer (EFFA, 2014).
01.061 1341	Undeca-1,3,5-triene		3795 16356-11-9	Liquid $C_{11}H_{18}$ 150.26	Slightly soluble Soluble	80-81 (16 hPa) NMR 94 %	1.510-1.518 0.788-0.796	Assay value: 94 % (54-56 % 3E,5Z-isomer, 35-37 % 3Z,5E-isomer and 3-5 % 3E,5E-isomer) and 1-3 % secondary

Table 3: Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2005a)

FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [(a)] Solubility in ethanol [(b)]	Boiling point, °C [(c)] Melting point, °C ID test Assay minimum	Refrac. Index [(d)] Spec.gravity [(e)]	EFSA comments
								component: 2,4,6-undecatriene (2Z,4Z,6E) (EFFA, 2014). CASrn in Register does not specify stereoisomers.
01.077 1344	1-Methyl-1,3-cyclohexadiene		1489-56-1	Liquid C_7H_{10} 94.16	Insoluble Soluble	118-120 NMR 95 %	1.446-1.452 0.846-0.853	

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

4. Genotoxicity Data

4.1. Genotoxicity Studies – Text Taken[13] from the JECFA (JECFA, 2006a)

Aliphatic and alicyclic hydrocarbons

In vitro

No evidence of mutagenicity was observed in Ames assays when camphene ([FL-no: 01.009]; up to 84,200 µg/plate), beta-caryophyllene ([FL-no: 01.007]; up to 150,000 µg/plate), *d*-limonene ([FL-no: 01.045]; up to 150,000 µg/plate), myrcene ([FL-no: 01.008]; up to 10,000 µg/plate), alpha-pinene ([FL-no: 01.004] (pin-2(3)-ene); up to 25,000 µg/plate), beta-pinene ([FL-no: 01.003] (pin-2(10)-ene); up to 5000 µg/plate), or p-mentha-1,4-diene ([FL-no: 01.020] (gamma-terpinene); up to 50,000 µg/plate) were incubated with *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537, TA1538, and/or UTH8413, UTH8414, or TA102 with and without metabolic activation (Rockwell and Raw, 1979; Florin et al., 1980; DeGraff, 1983; Haworth et al., 1983; Jagannath, 1984a; Jagannath, 1984b; Connor et al., 1985; Heck et al., 1989; NTP, 1990; Müller et al., 1993; NTP, 2004b; NTP, 2004c). Without metabolic activation, delta-3-carene [FL-no: 01.029] at doses of between 2157 and 4314 µg/plate gave positive results in the Ames assay in *S. typhimurium* strains TA100 and TA102, but gave negative results in both strains with metabolic activation (Kurttio et al., 1990). delta-3-Carene at doses of up to 4314 µg/plate also gave negative results in *S. typhimurium* strain TA98 with and without metabolic activation (Kurttio et al., 1990). In one Ames assay with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, the beta-isomer of cadinene (JECFA-no: 1346) gave negative results at doses of up to 10 000 and 3333 µg/plate, respectively, with and without metabolic activation (Haworth et al., 1983; NTP, 2004e). In another Ames assay, cadinene (isomer not specified) gave equivocal/weak positive results at doses of up to 10 000 µg/plate in *S. typhimurium* strains TA97 and TA100 with metabolic activation, but gave negative results at doses of up to 100 µg/plate in both strains without metabolic activation, as well as in strains TA98, TA1535 and TA1537 with and without metabolic activation (NTP, 2004d).

Camphene ([FL-no: 01.009]; 1.4–136.2 µg/ml), beta-caryophyllene ([FL-no: 01.007]; 2.0– 204.4 µg/ml), alpha-phellandrene ([FL-no: 01.006]; 4.5–136.2 µg/ml), and beta-pinene (FL-no: 01.003; 4.5–136.2 µg/ml) did not induce sister chromatid exchanges (SCE) in Chinese hamster ovary cells without metabolic activation (Sasaki et al., 1989).

Beta-caryophyllene ([FL-no: 01.007]; up to 10 000 µg/ml), alpha-pinene ([FL-no: 01.004]; up to 10 000 µg/ml), and p-mentha-1,4-diene ([FL-no: 01.020]; up to 30 µg/ml) did not induce unscheduled DNA synthesis in rat hepatocytes (Heck et al., 1989).

d-limonene [FL-no: 01.045] did not induce genetic effects when tested in *Saccharomyces cerevisiae* strain MP1, without metabolic activation, at concentrations of up to 230 mmol/l (Fahrig, 1984). In Chinese hamster ovary cells, *d*-limonene did not induce chromosomal aberrations at concentrations of 10 - 500 µg/ml, or SCE at concentrations of 1.4 - 162 µg/ml, with and without metabolic activation (Sasaki et al., 1989; Anderson et al., 1990; NTP, 1990). In an assay for forward mutation in mouse lymphoma cells, *d*-limonene produced negative results in L5178Y cells with and without metabolic activation, at a concentration of up to 100 µg/ml (Heck et al., 1989; Myhr et al., 1990; NTP, 1990). When incubated with Syrian hamster embryo cells, limonene (isomer not specified) did not induce cell transformation in one assay at a concentration of up to 100 µg/ml (Pienta, 1980), while in another assay concentrations up to 408.7 µg/ml increased the transformation frequency, albeit not in a statistically significant manner (Rivedal et al., 2000). The latter study also tested the effects of limonene (isomer not specified) on gap junction intercellular communication in Syrian hamster embryo cells. Limonene at concentrations of 1.4 - 136.2 µg/ml did not show effects (Rivedal et al., 2000).

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

With and without metabolic activation, myrcene [FL-no: 01.008] did not induce gene mutations at the hypoxanthine guanine phosphoribosyl transferase (*Hprt*) locus in Chinese hamster V79 cells, at concentrations of up to 1000 µg/ml (Kauderer et al., 1991), nor did it induce SCE in these cells at concentrations up to 500 µg/ml (Röscheisen et al., 1991). Myrcene also did not induce SCE or chromosomal aberrations in human lymphocytes, with and without metabolic activation, at concentrations of up to 1000 µg/ml (Kauderer et al., 1991), nor did it induce SCE in hepatic tumour cells, at concentrations of up to 500 µg/ml, although a slight, reproducible but not dose-dependent increase was noted (Röscheisen et al., 1991).

The beta-isomer of cadinene (No. 1346, not in Register) gave negative results in an assay for forward mutation in mouse lymphoma cells , at concentrations of up to 46.2 µg/ml without metabolic activation, and at up to 73.9 µg/ml with metabolic activation (NTP, 2004f). In Chinese hamster ovary cells, this β-isomer did not induce chromosomal aberration with or without metabolic activation at concentrations of 24.9 - 40 µg/ml, or SCE with metabolic activation at concentrations of up to 31.1 µg/ml (NTP, 2004g). Without metabolic activation, an equivocal result was obtained for induction of SCE, at concentrations up to 26.6 µg/ml (NTP, 2004f).

In a study conducted *in vivo-in vitro*, designed to investigate the mutagenicity of urinary metabolites of a number of food additives, Sprague-Dawley rats were given a single dose of 0.5 ml of camphene (FL-no: 01.009; approximately 1684 mg/kg bw) and α-pinene (FL-no: 01.004; approximately 1718 µg/kg bw) via gavage and the urine was collected for 24 h. Three types of urine sample were tested in the Ames assay with *S. typhimurium* strains TA98 and TA100 with metabolic activation: a direct urine sample, a urine-ether extract, and the aqueous fraction of the urine-ether extract. The urine samples of rats treated with α-pinene did not show any evidence of mutagenicity, either in the presence or absence of beta-glucuronidase. Of the urine samples of camphene-treated rats only the urine-ether extract showed a weak mutagenic response, and only in TA100, not in TA98 (Rockwell and Raw, 1979).

In vivo

In a mammalian spot test, no evidence of mutagenicity was observed in mouse C57BLxT embryos in utero after intraperitoneal injection of the dam with *d*-limonene [FL-no: 01.045] at a dose of 215 mg/kg bw per day on days 9 and 10 of gestation (Fahrig, 1984).

In an assay for cytogenetic changes in bone marrow, groups of Wistar rats (two or four of each sex per group) were given myrcene [FL-no: 01.008] at a dose of 100, 500, or 1000 mg/kg bw via gavage. A negative control group (two rats of each sex) received only the vehicle (corn oil) via gavage, while a positive control group (two rats of each sex) received cyclophosphamide at a dose of 30 mg/kg bw via intraperitoneal injection. A mitotic inhibitor (colchicine, administered at a dose of 5 mg/kg bw via intraperioneal injection) was administered 1 h before sacrifice at 24 or 48 h after treatment, at which time the bone-marrow cells were harvested. Compared with the negative control group, treatment with myrcene did not result in an increase of metaphase cells with chromosomal aberrations upon examination at 24 or 48 h. In contrast, in the positive control group chromosomal aberrations were found in 19 % of the bone-marrow metaphase cells examined. Although not clastogenic, myrcene caused a dose-dependent increase in the mitotic index in bone-marrow cells, indicating that it was present at a sufficient dose in the target tissue (Zamith et al., 1993).

An assay for micronucleus formation in mouse peripheral blood erythrocytes was performed, with samples of peripheral blood obtained within 24 h of the final exposure in a 13-week study of toxicity in which male and female $B6C3F_1$ mice were given myrcene [FL-no: 01.008] at a dose of up to 2000 mg/kg bw per day via gavage. Scoring of 1000 normochromatic erythrocytes (NCEs) for micronuclei revealed no increase in micronucleated NCEs at any dose (NTP, 2004h).

Conclusion on genotoxicity on aliphatic/alicyclic hydrocarbons

Seven substances in this group of flavouring agents have been tested in the Ames assay and found not to be mutagenic in bacteria *in vitro*. One flavouring agent, δ-3-carene, produced a positive result in this assay, only without metabolic activation, in *S. typhimurium* strains TA100 and TA102 but not TA98. Another flavouring agent, cadinene (isomer not specified), gave weakly positive results in the Ames assay, only with metabolic activation, in *S. typhimurium* strains TA97 and TA100 but not TA98, TA1535, and TA1537.

In mammalian cell systems, predominantly negative results were obtained for representative members of this group with respect to induction of SCE, chromosomal aberrations, unscheduled DNA synthesis, and gene mutations. In assays for cell transformation in Syrian hamster embryo cells, limonene (isomer not specified) gave negative results in one assay, but weak positive results in another, the increase in transformation frequency being not statistically significant.

Myrcene and *d*-limonene showed no signs of genetic toxicity in cytogenetic assays for micronucleus formation in bone marrow and peripheral erythrocytes (myrcene) and a mammalian spot test (*d*-limonene) performed *in vivo*.

On the basis of the results of available studies of genotoxicity, the Committee concluded that the flavouring agents in this group of aliphatic and alicyclic hydrocarbons are not genotoxic.

Aromatic hydrocarbons

In vitro

No evidence of mutagenicity was observed in standard or modified Ames assays when p-cymene ([FL-no: 01.002]; up to 85,300 µg/plate), was incubated with *Salmonella typhimurium* strains TA98, TA100, with metabolic activation (Rockwell and Raw, 1979).

In a study designed to investigate the mutagenicity *in vivo-in vitro* of urinary metabolites of a number of food additives, Sprague-Dawley rats were given 0.5 ml of p-cymene ([FL-no: 01.002]; approximately 1706 mg/kg bw) by gavage and urine was collected for 24 hours. Three types of urine samples were tested in the Ames assay with *S. typhimurium* strains TA98 and TA100 with metabolic activation: a direct urine sample, a urine-ether extract, and the aqueous fraction of the urine-ether extract. The urine samples of rats treated with p-cymene did not show any evidence of mutagenicity, either in the presence or absence of β-glucuronidase (Rockwell and Raw, 1979).

Conclusion on genotoxicity on aromatic hydrocarbons

One substance in this group of flavouring agents has been tested in the Ames assay and found not to be mutagenic *in vitro* in bacteria. On the basis of the results of available studies of genotoxicity, the Committee concluded that the flavouring agents in this group of aromatic hydrocarbons are not genotoxic.

For a summary of *in vitro/in vivo* genotoxicity data considered by the JECFA, see Tables 5 and 6.

4.2. Genotoxicity Studies – Text Taken[14] from EFSA FGE.25Rev3 (EFSA CEF Panel, in press)

Data from *in vitro* tests are available for two candidate substances [subgroup I: FL-no: 01.038 and FL-no: 01.057] and 10 supporting flavouring substances [one from subgroup II, four from subgroup III, and five from subgroup V (for pin-2(3)-ene [FL-no: 01.004] also data for separate stereoisomers were

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

available (+ and -)- α-pinene), and one structurally related substance, (2-methylbuta-1,3-diene) not used as flavouring substance from subgroup II. Data for three supporting substances [FL-no: 01.008 (subgroup II), FL-no: 01.019 (subgroup III), FL-no: 01.004 (subgroup V), and data for the structurally related substance from subgroup II are considered valid.

Data from *in vivo* tests are available for two supporting substances (one from *subgroup II* and one from *subgroup III*) and for one substance structurally related to *subgroup II* (2-methylbuta-1,3-diene).

Candidate substances

Subgroup I

The two candidate substances [FL-no: 01.038 and 01.057] tested *in vitro* for bacterial gene mutations gave negative results in bacterial reverse gene mutation tests and for mammalian cell gene mutations.

Subgroup II

For the three candidate substances in subgroup II [FL-no: 01.035, 01.064 and 01.070] there are no genotoxicity data available, but it was noted that in contrast to the structurally related substance 2-methyl-1,3-butadiene, these substances do not contain conjugated terminal double bonds, except [FL-no: 01.064].

The available *in vivo* studies on the structurally related substance 2-methylbuta-1,3-diene (isoprene) reported a negative result in a valid chromosomal aberration assay in the bone marrow of mice after 12 days of inhalatory exposure to isoprene. However, isoprene induced sister chromatid exchanges (SCE) in the bone marrow and micronuclei in peripheral blood cells of mice after 12 days of inhalatory exposure in two valid studies carried out within NTP. Induction of micronuclei in peripheral blood cells of mice has also been reported after inhalatory exposure for 13 weeks. In contrast, inhalatory exposure of isoprene to male and female rats for four weeks did not result in an increase in the frequency of micronuclei in the lung fibroblasts. The validity of the latter two studies cannot be evaluated due to limited details available. Isoprene has been reported to bind covalently to haemoglobin *in vivo* (IARC, 1999).

The genotoxic and carcinogenic potential of isoprene has been evaluated by IARC (1999a). It was concluded that there is sufficient evidence of carcinogenicity in experimental mammalians and that isoprene is *'possibly carcinogenic to humans' (Group 2B)* (IARC, 1999). Isoprene has been classified in the EU as a *'Muta. Cat. 3; R68'* and *'Carc. Cat. 2; R45'* (EFSA, 2004b).

The available data on *in vivo* genotoxicity of isoprene indicate a genotoxic potential of the substance. In the light of the evidence of carcinogenic activity of isoprene in rats and mice (NTP, 1999) and the genotoxic effects of isoprene in mice and the fact that the structurally related substance 1,3-butadiene is classified as a genotoxic carcinogen, the Panel concluded that there is reason for concern with respect to genotoxicity and carcinogenicity of isoprene. This substance has been deleted from the Register.

For the supporting substances myrcene, several *in vitro* genotoxicity tests and three *in vivo* genotoxicity studies were available. All the *in vitro* genotoxicity tests on myrcene were negative. Two micronucleus tests on peripheral blood cells and one chromosomal aberration assay with myrcene gave negative results.

Conclusion on Genotoxicity for subgroup II

The supporting substance myrcene [FL-no: 01.008], which by the Panel is considered a more adequate supporting substance for the substances in subgroup II, has like isoprene, two conjugated terminal double bonds but has a longer chain length, with 10 carbon atoms, like [FL-no: 01.064]. The

genotoxicity data available on myrcene do not give rise to concern with respect to genotoxicity. Therefore, the Panel has not concern for genotoxicity for the three substances in subgroup II.

Subgroup III

For the five candidate substances in subgroup III no genotoxicity studies were available. For the four supporting substances, *d*-limonene [FL-no: 01.045], gamma-terpinene [FL-no: 01.020], α-terpinene [FL-no: 01.019] and α-phellandrene [FL-no: 01.006], several *in vitro* studies on genotoxicity were available and they were all negative. Also two *in vivo* Comet assay with *d*-limonene and a study with *d*-limonene in BigBlue™ rats were found negative. Therefore, the Panel has no concern for genotoxicity for the substances in subgroup III.

Subgroup V

For the candidate substance in subgroup V there are no genotoxicity data available. For the supporting substances, only negative results were reported in the available studies except for delta-3-carene (see Table 7). Delta-3-carene was studied individually as a component in wood fumes and wood fume condensates. A bacterial reverse gene mutation study (insufficiently reported) showed that delta-3-carene induced gene mutations in TA100 and TA102 strains in the absence of metabolic activation at high concentrations only, while it was negative in the presence of metabolic activation (Kurttio et al., 1990).

Information on the supporting substance β-caryophyllene [FL-no: 01.007] has been provided by EFFA (EFFA, 2012). The new data submitted cover a bacterial reverse mutation assay and an *in vivo* mouse erythrocyte micronucleus test.

In vitro

No evidence of genotoxic potential was observed when *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2uvrA were incubated with five test concentrations between 2300 and 9000 µg/plate in the presence or absence of rat liver (S9) bioactivation system using the plate incorporation method (Di Sotto et al., 2008). The positive and negative controls provided the appropriate response in the tester strains. However, the study design and reporting exhibits major deviations from OECD guideline 471 and is considered of insufficient quality.

In vivo

In an *in vivo* micronucleus induction assay, groups of mice (National Institute of Hygiene, Mexico) (5/sex/dose) were administered a single dose of 0, 20, 200 and 2000 mg/kg bw of β-caryophyllene by corn oil gavage. Blood was drawn and smears for analysis were prepared at 24, 48, 72 and 96 hours post dose. No significant increase in the induction of micronucleated polychromatic erythrocytes (MNPE) was observed for the treatments groups while all positive controls provided the appropriate response (Molina-Jasso et al., 2009). In a follow up study groups of the same strain of mice (5/sex/dose) were administered daily doses of 0, 20, 200 and 2000 mg/kg bw for three consecutive days by corn oil gavage with blood sampled and smears for analysis prepared at 24, 48, 72 and 96 hours post administration. There was no significant increase in MNPE however there was a slight increase in MNPE at the highest dose tested from 48 - 96 hours post dose. The authors did not consider this an indication of genotoxic potential due to the high dose administered over three consecutive days (Molina-Jasso et al., 2009). The Panel noted that the limit dose (2000 mg/kg) was applied in both treatment regimens without signs of toxicity (altered PCE/NCE ratio). The study is compliant with OCED guideline 474, except the reporting of individual data and historical controls; therefore, this study is considered of limited validity.

Altogether, the Panel has no concern for genotoxicity for the substances in subgroup V.

Data on genotoxicity are summarised in Table 7 and 8.

Data on the genotoxicity of the flavouring substances in this group are limited and the genotoxicity could not be assessed adequately for these substances. However, the Panel concluded that the available data do not preclude evaluating the 14 candidate substances using the Procedure.

4.3. New Mutagenicity/Genotoxicity Studies on β-Caryophyllene [FL-no: 01.007]

Information on the representative substance β-caryophyllene [FL-no: 01.007] has been provided by EFFA (EFFA, 2012). The new data submitted cover a bacterial reverse mutation assay and an *in vivo* mouse erythrocyte micronucleus test.

In vitro

No evidence of genotoxic potential was observed when *S. typhimurium* strains TA98 and TA100 and *E. coli* WP2uvrA were incubated with 5 test concentrations between 2300 and 9000 µg/plate in the presence or absence of rat liver (S9) bioactivation system using the plate incorporation method (Di Sotto et al., 2008). The positive and negative controls provided the appropriate response in the tester strains. However, the study design and reporting exhibits major deviations from OECD guideline 471 (see Table 9).

In vivo

In an *in vivo* micronucleus induction assay, groups of mice (5/sex/dose; mouse strain from National Institute of Hygiene (NIH), Mexico) were administered by gavage as a single dose of 0, 20, 200 and 2000 mg/kg bw of β-caryophyllene solved in corn oil. Blood was drawn and smears for analysis were prepared at 24, 48, 72 and 96 hours post dose. No significant increase in the induction of micronucleated polychromatic erythrocytes (MNPE) was observed for the treatment groups while all positive controls provided the appropriate response (Molina-Jasso et al., 2009). In a follow up study groups of NIH mice (5/sex/dose) were administered daily doses of 0, 20, 200 and 2000 mg/kg bw for three consecutive days by gavage with blood sampled and smears for analysis prepared at 24, 48, 72 and 96 hours post administration. The Panel noted that the limit dose (2000 mg/kg) was applied in both treatment regimens without signs of toxicity (altered reticulocyte/NCE ratio). A slight, non-significant increase in the MNPE frequency was observed in the highest dose group at 48, 72 and 96 hours, respectively. However, at none of these time points the effects were clearly dose-related since the low and medium doses resulted in lower MNPE frequencies than that observed in control animals. Overall, the Panel concluded that β-caryophyllene did not cause a significant increase in MNPE. However, the study exhibits deviations from OECD guideline 474 and therefore, is considered to be of limited validity (see Table 9).

Although the newly submitted data are of limited validity they do not preclude the substances to be evaluated using the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data on β-caryphyllene, see Table 9.

4.4. EFSA Considerations

The Panel agrees with the JECFA conclusions on genotoxicity.

5. Data Submitted Since Publication of FGE.78Rev1

A 90-day study requested in the previous version of this FGE was submitted for ß-caryophyllene [FL-no: 01.007] (Bauter, 2013a) along with a 14-day study (Bauter, 2011).

5.1. 14-Day Study on β-Caryophyllene [FL-no: 01.007]

In a 14 day range finding dietary study (Bauter, 2011), groups (3/sex/dietary intake level) of male and female Hsd:SD® rats were fed a diet designed to provide 0 (dietary control), 6000, 18 000 and 48 000 mg/kg feed of β-caryophyllene daily. These estimated dietary levels correspond to the measured intake of 0, 516, 1547 and 3569 mg/kg bw per day for males and 0, 528, 1582 and 4438 mg/kg bw per day for females, respectively. Clinical observations were recorded daily and body weights and food consumption observations were made on days 0, 7 and 14. There were no mortalities. Hyperactivity observed in 1/3 (33 %) of males and females in group 4 (48 000 mg/kg feed) in the latter part of the study may be possibly attributed to test substance administration. Dose-dependent decreases in male food consumption and food efficiency with significant corresponding decreases in group 4 male body weight and body weight gain were considered a result of test substance administration, but were not correlated with any other clinical signs. Females did not exhibit significant differences from female control. Findings at terminal sacrifice included all (100 %) males and females of group 4 with distention (cecum) and slight redness of the stomach, small intestines, and cecum. Based upon the limited toxicological endpoints evaluated, the study authors selected doses for the 90-day study.

5.2. 90-Day Study on β-Caryophyllene [FL-no: 01.007]

In an OECD 408 compliant 90-day study, 4 groups of rats (10/sex/dietary intake level) of male and female CRL Sprague-Dawley CD®IGS rats were fed a diet designed to provide 0 (dietary control), 3500, 7000 and 21 000 mg/kg feed and 3500, 14 000 and 56 000 mg/kg feed of β-caryophyllene for males and females, respectively, daily (Bauter, 2013a). These dietary levels corresponded to measured daily intakes of 0, 222, 456 and 1367 mg/kg bw for males and 0, 263, 1033 and 4278 mg/kg bw for females, respectively (Bauter, 2013a). The purity of the β-caryophyllene preparation was between 99 % (start of study) and 96 % (end of study). Clinical observations of toxicity were performed on day 0 and weekly until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed during week 12 after overnight fast. Urine was collected during the 15 hours prior to the blood draw. Prior to initiation of the study and on day 91 the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. At termination of the study all survivors were sacrificed and subject to full necropsy.

There were no mortalities, clinical signs of toxicity or ophthalmological changes associated with the presence of β-caryophyllene in the diet. There were statistically significant and concentration-related reductions in body weight gain, food consumption and food efficiency in males and females at the 21 000 mg/kg feed and 56 000 mg/kg feed concentration groups, respectively (body weight of high dose groups: males 77.3 %, females 82.6 % as compared to controls).

Although for some parameters statistically significant differences were found when compared to concurrent controls, haematology, clinical chemistry, coagulation and urine analysis parameters for the middle and high concentrations for both males in general were within the range of historical controls. Most of these changes were small, and observed in the highest dose group. Thus, in the female test groups, a statistically significant increase for platelet count was reported at the highest dietary level; such an increase was not observed in the male test groups. A dose dependent increase in white blood cells in males reached statistical significance at the middle and high dose; several other blood cells showed significant changes at the highest dose as well in males; the effects in females were less pronounced. There were no histopathology findings correlating to these variations.

In females a dose-dependent decrease in serum glucose concentrations and an increase in triglyceride levels reached statistically significance only at the highest dose level. In conjunction with changes reported in the liver these changes were attributed to metabolic changes as a result of high concentrations of β-caryophyllene in the diet. Pathological findings include increases in absolute and relative liver weights; these were found statistically significant in the mid- and high-dose groups of both sexes. Histopathological liver changes at the mid and high intake levels for both sexes were characterized by centrilobular to midzonal distributed hepatocellular hypertrophy. Based on

hepatocyte hypertrophy in both sexes, the increases in absolute and/or relative liver weights at the mid- and highest dietary levels, and the absence of any other significant abnormality upon histopathological examination, the study authors conclude that the hepatocyte hypertrophy is the result of hepatic enzyme induction; this has, however, not been confirmed by measurements of relevant enzymes.

Necropsy revealed enlarged kidneys in one male at the highest test concentration and significant increases in relative kidney weight of male high-dose as well as of female mid- and high-dose groups. Microscopic examination revealed mononuclear infiltration in only one of ten females of the highest dose group. No other microscopically visible alterations were reported in the kidneys of female rats. The increase in kidney weights in female remains unexplained. Microscopical examination of the kidney of males revealed an increased incidence of nephropathy characterised by regeneration of proximal cortical tubules with thickened membranes, mononuclear cell infiltration and tubular casts at all dose levels. The severity of the nephropathy exhibited a dose-dependent shift from low to high grades. Kidney cells of affected males also were reported to have necrotic nuclei and an increase in eosinophilic cytoplasm. Likewise, in the kidneys of all treated males eosinophilic cytoplasmic droplets were present, with a dose-dependent shift to higher grades. In a supplementary study the kidney slides of exclusively male rats were stained also with Mallory Heidenhain stain (Zook and Garlick, 2013), which results in enhanced staining of the cytoplasmic droplets (De Rijk et al., 2003; Frazier et al., 2012); this confirmed the observations of the eosine staining. An increased hyaline droplet accumulation in male rats exclusively is characteristic of α_{2u}-globulin nephropathy (Hard et al., 1993), which is considered a male rat specific effect with little relevance for humans. Although no specific immunohistochemical staining of α_{2u} –globulin has been done to confirm the presence of this protein, the Panel considers the evidence sufficient to conclude that this kidney toxicity in male rats exclusively is not relevant for humans.

Microscopic examination of the mesenteric lymph nodes revealed the presence of erythrocytes in the sinuses at the mid- and high-intake levels for both sexes. Additionally, reduced spleen weights for males at the highest dietary level were considered related to general reductions in lymphoid system weights.

The Panel concluded that under the conditions of the present 90-day dietary toxicity study and based on the toxicological findings in haematology in males, the liver, the mesenteric lymph node pathology in both sexes and non-explained effects in female kidneys only the lowest dose provides a no-adverse-effect level (NOAEL) for β-caryophyllene, which is the lowest in male rats: 222 mg/kg bw per day.

5.3. 90-Day Study on Myrcene [FL-no: 01.008]

90-Day Study by the NTP (2010); Administration by Gavage.

Groups of 10 male and 10 female F344/N rats were administered 0, 250, 500, 1000, 2000 or 4000 mg myrcene/kg body weight in corn oil by gavage, five days per week for 14 weeks. Additional groups of 10 male and 10 female special study rats were administered the same doses for 23 days (NTP, 2010). The results of this study have been summarised in more detail in FGE 25Rev3.

All rats in the 4000 mg/kg bw groups died. One to three rats in the 1000 and 2000 mg/kg groups and one 500 mg/kg bw male died by week 10 of the study. Mean body weights were significantly decreased in male rats in the 500, 1000, and 2000 mg/kg bw groups. Right kidney and liver weights of dosed males and females were generally significantly greater than those of the vehicle controls. In special study rats evaluated on day 23, the incidences and severities of chronic progressive nephropathy (CPN) and renal tubule degeneration were increased in 2000 mg/kg bw males. At the end of the 3-month study, the incidences of renal tubule necrosis were significantly increased in all dosed groups of males and females. Of the core control males 7 out of 10 showed nephropathy at the end of the study; in the low dose group this was 10/10. For females 1/10 controls showed nephropathy while for the low dose group this was 2/10. In all cases the nephropathy was judged to be minimal. At 3

months, the incidences of olfactory epithelium degeneration in 2000 mg/kg bw males and females were significantly increased, and the severities were increased. The incidences of chronic inflammation in 1000 and 2000 mg/kg bw males and females were significantly increased. All 2000 mg/kg bw males and females had splenic atrophy. In the mesenteric lymph node, significantly increased incidences of atrophy occurred in 2000 mg/kg bw males and 1000 and 2000 mg/kg bw females. Acute inflammation of the forestomach occurred in four 2000 mg/kg bw females. The incidences of porphyrin pigmentation in the Harderian gland of males administered 500 mg/kg bw or greater were significantly increased.

The results indicate that at the lowest dose of 250 mg myrcene/kg bw by gavage all females and males showed renal tubule necrosis. The severity of the lesion was judged to be minimal; it increased with the dose to mild/moderate. These results were confirmed in a 2 year study in rats by gavage of the same dose. No NOAEL could be established from this study.

90-Day Study by Bauter (2013b); Administration in the Feed.

In an OECD 408 compliant 90-day study the subchronic toxicity of myrcene (93.3 % pure) was evaluated in male and female rats, based on continuous exposure to the test substance in the diet (Bauter, 2013b). Four groups of adult Sprague-Dawley rats (10/sex/group) were maintained on diets prepared to contain 0, 700, 2100, or 4200 mg/kg feed of myrcene. However, as explained below, the myrcene content of the diet decreased considerably over 7 days, so that every week a new charge of the diet was prepared. Therefore, the Panel decided to take the concentration on the last day of the week for quantification of the exposure, rather than the (logarithmic) mean over the whole week. Based on the stability data, weekly diet refreshment, and measured dietary intake, an adjusted calculated mean daily intake of 8.0, 40 , and 44 mg/kg bw per day for males, and 9.6, 48 and 53 mg/kg bw per day for females for 90 days was calculated.

The neat test substance was measured to be stable under the conditions of storage over the course of this study. Stability of test substance in the diet was evaluated by analysing dietary levels of myrcene on days 0, 4, 7, and 10 following preparation, as well as daily, for seven days, in an independent stability assessment. At the beginning, middle, and end of the study, diet preparation samples were analysed to verify the concentration of myrcene in the diet over the course of the study.

The myrcene content at the 700 mg/kg feed level was found to decrease from 72 to 18 % from day 0 to day 7, with an average intake of 45 % of the target concentration over the course of the study. Similarly, the myrcene content at the 2100 mg/kg feed level decreased from 65 to 30 %, with an average intake of 44 % of the target concentration over the course of the study. The myrcene content at the 4200 mg/kg feed level decreased from 69 to 17 % from day 0 to day 7, with an average intake of 43 % of the target concentration over the course of the study.

Prior to study initiation and again on day 88, the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. The animals were observed for viability, signs of gross toxicity and behavioural changes at least once daily during the study and weekly for a battery of detailed clinical observations. Body weights were recorded twice during acclimation, including prior to test initiation (day 0) and together with food consumption, weekly thereafter and prior to terminal sacrifice. Blood and urine samples were collected on day 89 from all surviving study animals for urinalysis, hematology, and clinical chemistry determinations. Coagulation assessments were performed on day 92 or 93, preceding necropsy. Gross necropsies were performed on all study animals and histological evaluation of selected organs and tissues from control animals (0 mg/kg feed), animals administered the highest dietary level, and tissues with gross macroscopic observations.

There were no mortalities, clinical, or ophthalmological changes attributable to myrcene administration. There were no statistically significant, dietary concentration-dependent changes in body weight, body weight gain, food consumption, or food efficiency in males and females attributed to the administration of myrcene during the study.

There were no clinical pathological findings, changes in macroscopic or microscopic histopathology, or organ weight changes in the groups administered myrcene. Some incidental changes in clinical chemistry and hematology parameters were within approximate historical control values, did not correlate with macroscopic or histopathological findings, were without biologic impact, and were considered not toxicologically relevant. A few histopathological changes were considered incidental, spontaneous in nature as observed for the age and strain of rat used in this study, and had no established relationship to administration of the test substance.

Under the conditions of the study and based on the toxicological endpoints evaluated, the no-adverse-effect level (NOAEL) for administration of myrcene in the diet was determined to be the highest dose-group, calculated to provide an estimated daily intake of 44 mg/kg bw per day for males and 53 mg/kg bw per day for females, respectively.

The Panel took note of the fact that two 90-day studies are available on myrcene: the NTP study and the Bauter study. While the NTP study showed kidney toxicity in all animals at 250 mg/kg bw per day, the Bauter study observed no toxicity at all in the kidneys at 44 and 53 mg/kg bw per day in males and females resp. The Panel decided to accept the NOAEL of the Bauter study (44 mg/kg bw per day) because:

- the NTP study is a gavage study, which leads to higher exposure levels compared to the Bauter study in which the compound was added in the feed,
- the Bauter study reflects the dietary administration (consumption with food matrix, over a more extended period of time) in consumers much better than gavage (NTP) study,
- in the Bauter study no kidney toxicity was observed,
- the NOAEL derived from the Bauter study is six-fold lower than the effect level in the NTP study.

6. Application of the Procedure

6.1. Application of the Procedure to 22 Aliphatic, Alicyclic and Aromatic Hydrocarbons by the JECFA (JECFA, 2006a)

Aliphatic and alicyclic hydrocarbons

According to the JECFA all 19 aliphatic and alicyclic substances belong to structural class I using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded 16 aliphatic and alicyclic hydrocarbons at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural class I (step A3). For three substances, *d*-limonene, myrcene and α-pinene [FL-no: 01.045, 01.008 and 01.004] the estimated daily intake exceeds the threshold for structural class I and these substances were evaluated at step A5.

For myrcene [FL-no: 01.008] a lowest-observed-effect level (LOEL) of 250 mg/kg bw per day was reported for male mice and male and female rats treated by gavage for 13 weeks (NTP, 2004a), while the same dose was the no-observed-effect level (NOEL) in female mice. This dose is approximately 1800 times greater than the estimated intake of myrcene from its use as a flavouring agent in Europe (140 µg/kg bw per day) and 83 000 times greater than the estimated intake of myrcene in the USA (3 µg/kg bw per day). The Committee concluded that myrcene would not pose a safety concern at estimated current intake.

At its 41st meeting, the Committee established an ADI 'not specified' for *d*-limonene [FL-no: 01.045] on the basis of short- and long-term studies of toxicity in female rats and male and female mice, and

studies of developmental toxicity in mice, rats and rabbits. In these studies, *d*-limonene was tested at doses ranging from 250 to 2800 mg/kg bw per day. Based on the ADI 'not specified', the Committee concluded that *d*-limonene would not pose a safety concern at the estimated current intakes (660 µg/kg bw per day in Europe and 210 µg/kg bw per day in the USA).

No toxicological data on α-pinene [FL-no: 01.004] were available. *d*-Limonene shares structural characteristics with α-pinene in that both contain a methyl-substituted cyclohexene ring, which contains a second alkyl substituent. In *d*-limonene, this is an isopropenyl group, while in α-pinene the second substituent is a dimethyl-substituted methylene bridge. Based on these chemical structures, it would be predicted that the toxicity of α-pinene would be unlikely to exceed that of *d*-limonene. Both compounds are predicted to be metabolised to innocuous products. Metabolism of both compounds is by hydroxylation of the cyclohexene ring and oxidation of its methyl substituent. *d*-Limonene undergoes epoxidation of the endocyclic and allylic double bonds, leading to dihydroxy products. α-Pinene is converted to several metabolites, including *d*-limonene, by rat liver microsomes *in vitro*. The Committee concluded that *d*-limonene shared sufficient chemical and metabolic similarities with α-pinene to be used as a structural analogue for α-pinene at this step of the Procedure. The estimated current per capita intakes of α-pinene in Europe (36 µg/kg bw per day) and in the USA (41 µg/kg bw per day) are approximately 5 % and 20 %, respectively, of those of *d*-limonene, and are almost four orders of magnitude lower than the lowest doses of *d*-limonene considered in the establishment of its ADI 'not specified'. On the basis of these considerations, the Committee concluded that α-pinene would not pose a safety concern at estimated current intakes.

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

Aromatic hydrocarbons

According to the JECFA [FL-no: 01.002 and 01.010] belong to structural class I using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded both aromatic hydrocarbons at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and have intakes below the thresholds for their structural class I (step A3).

In conclusion, the JECFA evaluated aromatic hydrocarbons with [FL-no: 01.002 and 01.010] as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the in total 21 aliphatic and alicyclic hydrocarbons and aromatic hydrocarbons are summarised in Table 11: Summary of Safety Evaluation by the JECFA (JECFA, 2005b).

6.2. Application of the Procedure to Aliphatic Hydrocarbons in FGE.25Rev3 (EFSA CEF Panel, in press)

For the safety evaluation of the 14 candidate substances from chemical group 31, the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of these 14 substances are summarised in Table 12.

Step 1

All 14 candidate substances evaluated using the Procedure are classified into structural class I [FL-no: 01.001, 01.027, 01.028, 01.033, 01.034, 01.035, 01.038, 01.039, 01.046, 01.054, 01.057, 01.059, 01.064 and 01.070], according to the decision tree approach presented by Cramer *et al*. (Cramer et al., 1978).

Step 2

On the basis of the metabolism information available, five of the six candidate substances of subgroup I [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] and five of the six candidate substances of subgroup III [FL-no: 01.001, 01.027, 01.028, 01.039 and 01.046] (see Table 4) may be predicted to be metabolised to innocuous products at the estimated levels of intake based on the MSDI approach, and accordingly the evaluation of these 10 substances proceeds along the A-side of the Procedure scheme. For the remaining two candidate substances [FL-no: 01.035 and 01.059] there are not sufficient data available on biotransformation to conclude that they will be metabolised to innocuous products, and therefore their evaluation will proceed along the B-side of the Procedure scheme.

Two candidate substances from subgroup II [FL-no: 01.064 and 01.070] contain terminal double bonds in the absence of other functional groups that may provide alternative routes of detoxication. Therefore, for these two substances it cannot be concluded that they will be metabolised to innocuous products, and accordingly they proceed along the B-side of the Procedure scheme.

Step A3

The five candidate substances from subgroup I [FL-no: 01.033, 01.034, 01.038, 01.054 and 01.057] and the three candidate substances from subgroup III [FL-no: 01.027, 01.028 and 01.039], proceeding via the A-side, have been assigned to structural class I and have estimated European daily *per capita* intakes ranging from 0.012 to 2.7 µg (Table 12). These intakes are below the threshold of concern of 1800 µg/person per day for structural class I. Accordingly, it is concluded that these eight candidate substances do not pose a safety concern as flavouring substances when used at estimated levels of intake, based on the MSDI approach. The two candidate substances from subgroup III [FL-no: 01.001 and 01.046] have an estimated European daily *per capita* intake of 4000 and 2100, respectively, which are above the threshold of concern of 1800 µg/person per day for structural class I. The evaluation of these candidate substances will therefore proceed to A4 of the Procedure.

Step A4

The candidate substances [FL-no: 01.001 and 01.046] or its metabolites are not endogenous.

Step A5

The two candidate substances [FL-no: 01.001 and 01.046] are supported by the substance [FL-no: 01.045] for which an adequate carcinogenicity study is available. From this study a no observed adverse effect level (NOAEL) of 215 mg/kg bw per day can be derived. The estimated daily *per capita* intake is 4000 µg for [FL-no: 01.001] and 2100 µg for [FL-no: 01.046], corresponding to 0.07 mg/kg bw per day and 0.035 mg/kg bw per day at a body weight of 60 kg, respectively. Thus, a margin of safety of 3070 can be calculated for [FL-no: 01.001] and a margin of safety of 6140 can be calculated for [FL-no: 01.046]. These two substances are accordingly not expected to be of safety concern at the estimated levels of intake.

Step B3

The four candidate substances [FL-no: 01.035, 0.059, 01.064 and 01.070] proceeding via the B-side and which have been assigned to Cramer structural class I have estimated European daily *per capita* intakes between 0.0085 and 14 µg (Table 12). These intakes are below the threshold of concern of 1800 µg/person per day for structural class I. Accordingly, these four substances all proceed to step B4 of the Procedure.

Step B4

For one of these substances, 4(10)-thujene [FL-no: 01.059] a margin of safety could be calculated based upon a NOAEL (222 mg/kg bw) for the supporting substance ß-caryophyllene [FL-no: 01.007]. Compared to the MSDI of 4(10)-thujene of 14 µg/*capita* per day equal to 0.2 µg/kg bw per day, the NOAEL provides a margin of safety of 9.5×10^5.

For the three remaining substances, 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] a margin of safety could be calculated based upon a NOAEL (44 mg/kg bw) for the supporting substance myrcene [FL-no: 01.008]. Compared to the MSDI of 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] and 1-octene [FL-no: 01.070] of 9.1, 14 and 0.0085 µg/*capita* per day equal to 0.15, 0.23 and 0.00014 µg/kg bw per day, the NOAEL provides a margin of safety of 2.9×10^5, 1.9×10^5 and 3.1×10^8.

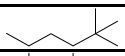
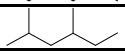
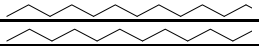
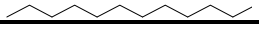
The stepwise evaluations of the 14 substances are summarised in Table 12: Summary of Safety Evaluation by the EFSA (FGE.25Rev3).

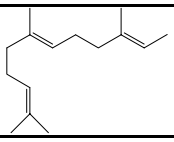
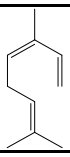
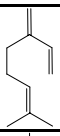
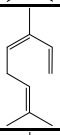
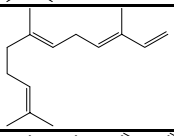
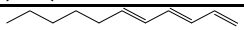
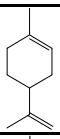
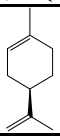
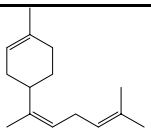
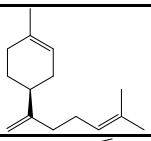
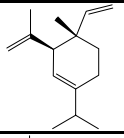
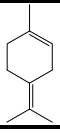
6.3. EFSA Considerations

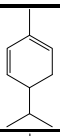
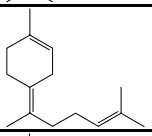
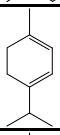
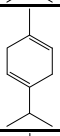
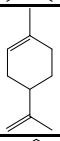
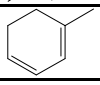
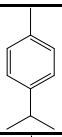
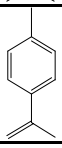
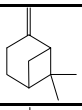
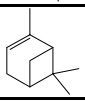
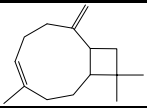
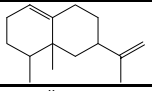
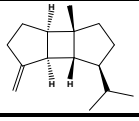
In FGE.78Rev1, the information on myrcene came from FGE.25Rev2, in which data on myrcene have been evaluated in detail. Based on the toxicity study of myrcene, available at that time, it was concluded in FGE.25Rev2 that: "No overall NOAEL from the NTP study on myrcene could be allocated due to the observation renal toxicity in male and female rats at all dose groups. The Panel has considered deriving a BMDL (benchmark dose level) from the NTP study of myrcene. However, a BMDL from this study could not be derived since nearly 100 % incidence of nephropathy was observed in male rats already at the lowest dose of myrcene. Since then, new toxicity data have become available (Bauter, 2013b) and a no-adverse-effect level (NOAEL) for administration of myrcene in the diet to rats was calculated to provide an estimated daily intake of 44 mg/kg bw per day for males and 53 mg/kg bw per day for females, respectively.

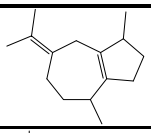
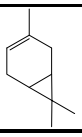
The 21 JECFA evaluated hydrocarbons and the 14 EFSA evaluated hydrocarbons are distributed into subgroups of structurally related substances by EFSA. The Panel conclusions on predictions of metabolism are shown in Table 4 below.

Table 4: Subgroups as Classified by EFSA within the Groups of the JECFA evaluated Hydrocarbons (JECFA, 2006a) and the EFSA evaluated Hydrocarbons in FGE.25Rev3 (EFSA CEF Panel, in press). The JECFA evaluated Hydrocarbons are Listed in Brackets.

FL-no	EU Register name	Structural formula	Struc. class	Predicted metabolism to innocuous products? (by EFSA)	A- or B-side of the Procedure (EFSA)	A- or B- side of the Procedure (JECFA)
I: ACYCLIC ALKANES						
01.033	2,2-Dimethylhexane		I	Yes	A	
01.034	2,4-Dimethylhexane		I	Yes	A	
01.038	Dodecane		I	Yes	A	
01.054	Pentadecane		I	Yes	A	
01.057	Tetradecane		I	Yes	A	

II: ACYCLIC ALKENES						
01.035	2,6-Dimethylocta-2,4,6-triene		I	No (lack of supporting data)	B	
01.064	cis-3,7-Dimethyl-1,3,6-octatriene		I	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)	B	
01.070	1-Octene		I	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)	B	
(01.008)	(Myrcene)		I	No (lack of supporting data)	B	A
(01.018)	(β-Ocimene)		I	No (lack of supporting data)	B	A
(01.040)	(α-Farnesene)		I	No (lack of supporting data)	B	A
(01.061)	(Undeca-1,3,5-triene)		I	No (lack of supporting data)	B	A
III: CYCLOHEXENE HYDROCARBONS						
01.001	Limonene		I	Yes	A	
01.046	l-Limonene		I	Yes	A	
01.027	Bisabola-1,8,12-triene		I	Yes	A	
01.028	β-Bisabolene		I	Yes	A	
01.039	δ-Elemene		I	Yes	A	
(01.005)	(Terpinolene)		I		A	A

(01.006)	(α-Phellandrene)		I		A	A
(01.016)	(1,4(8),12-Bisabolatriene)		I		A	A
(01.019)	(α-Terpinene)		I		A	A
(01.020)	(gamma-Terpinene)		I		A	A
(01.045)	(d-Limonene)		I		A	A
(01.077)	(1-Methyl-1,3-cyclohexadiene)		I		A	A
IV: AROMATIC HYDROCARBONS						
IVb: NAPHTHALENE HYDROCARBONS						
IVe: ALKYL SUBSTITUTED BENZENE HYDROCARBONS						
(01.002)	(1-isopropyl-4-methylbenzene)		I		A	A
(01.010)	(1-isopropenyl-4-methylbenzene)		I	No	B	A
V: BI- and TRICYCLIC, NONAROMATIC HYDROCARBONS						
01.059	4(10)-Thujene			No (but supported by 01.007)	B	
(01.003)	(Pin-2(10)-ene)		I	No (but supported by 01.007)	B	A
(01.004)	(Pin-2(3)-ene)		I	No (but supported by 01.007)	B	A
(01.007)	(β-Caryophyllene)		I	No (but supported by 01.007)	B	A
(01.009)	(Camphene)		I	No (but supported by 01.007)	B	A
(01.017)	(Valencene)		I	No (but supported by 01.007)	B	A
(01.024)	(β-Bourbonene)		I	No (but supported by 01.007)	B	A

(01.026)	(1(5),7(11)-Guaiadiene)		I	No (but supported by 01.007)	B	A
(01.029)	(δ-3-Carene)		I	No (but supported by 01.007)	B	A

The Panel agrees with the application of the Procedure as performed by the JECFA for seven substances in the subgroup III (Cyclohexene hydrocarbons) and one substance in subgroup IVe (Alkyl substituted benzene hydrocarbons) [FL-no: 01.002, 01.005, 01.006, 01.016, 01.019, 01.020, 01.045 and 01.077], which all are evaluated via the A-side of the Procedure with the conclusion "no safety concern at estimated levels of intake based on the MSDI approach".

For the remaining 14 substances which were all predicted to be metabolised to innocuous products (evaluated via the A-side of the Procedure) by the JECFA the Panel does not agree with the way the JECFA applied the Procedure for the evaluation of Flavouring Substances.

For four substances in subgroup II [FL-no: 01.008, 01.018, 01.040 and 01.061]: Acyclic alkenes, it could not be anticipated that the substances would be metabolised to innocuous products due to presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options. In a 90 day study in rats by Bauter (Bauter, 2013b) on the representative substance myrcene [FL-no: 01.008] a NOAEL of 44 mg/kg bw per day could be established. Compared to the MSDI of myrcene of 290 µg/*capita* per day equal to 4.8 µg/kg bw per day, the NOAEL provides a margin of safety (MOS) of 9200. The MOS for [FL-no 01.018, 01.040 and 01.061] based upon the NOAEL for myrcene are $4.8x10^5$, $4.4x10^6$ and $1.1x10^7$, respectively. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be evaluated at step B4 in the Procedure as being of no safety concern.

For one substance (1-isopropenyl-4-methylbenzene, [FL-no: 01.010]) in subgroup IVe: Alkyl substituted benzenes, it could not be anticipated that the substance would be metabolised to innocuous products due to presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options. In a 90 day study in rats by Posternak et al. (Posternak et al., 1969) NOAEL 0.625 mg/kg bw per day could be established. Compared to the MSDI of 18 µg/*capita* per day ~ 0.3 µg/kg bw per day, the NOAEL provides a margin of safety of 2000.

For eight substances in subgroup V [FL-no: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026 and 01.029]: Bi- and tricyclic, nonaromatic hydrocarbons, no data are available to conclude that the substances will be metabolised to innocuous products. In a 90 day study in rats by Bauter (Bauter, 2013a) on the representative substance ß-caryophyllene [FL-no: 01.007] a NOAEL of 222 mg/kg bw per day could be established. Compared to the MSDI of ß-caryophyllene of 330 µg/*capita* per day equal to 5.5 µg/kg bw per day, the NOAEL provides a margin of safety of 40 000. The margin of safety for [FL-no 01.003, 01.004, 01.009, 01.017, 01.024, 01.026 and 01.029] based upon the respective MSDI for these substances and the NOAEL for ß-caryophyllene, range between 7400 and $1.1x10^7$. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be evaluated at step B4 in the Procedure as being of no safety concern.

CONCLUSION

The present Revision of FGE.78, FGE.78Rev2, includes the assessment of additional available toxicity data for β-caryophyllene [FL-no: 01.007] and myrcene [FL-no: 01.008]. The data provided are for both substances a 90-day study. Furthermore, new short term study and genotoxicity data have been provided for [FL-no: 01.007].

The Panel concluded that the 21 substances in the JECFA flavouring groups of aliphatic and alicyclic and aromatic hydrocarbons are structurally related to the group of 14 aliphatic hydrocarbons evaluated by EFSA in the Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3).

The Panel agrees with the application of the Procedure as performed by the JECFA for eight of the 21 substances considered in this FGE [FL-no: 01.002, 01.005, 01.006, 01.016, 01.019, 01.020, 01.045 and 01.077].

For the following 13 substances [FL-no: 01.003, 01.004, 01.007, 01.008, 01.009, 01.010, 01.017, 01.018, 01.024, 01.026, 01.029, 01.040 and 01.061] it cannot be concluded that they are metabolised to innocuous substances and therefore their evaluation should proceed along the B-side of the Procedure. For one of these substances, 1-isopropenyl-4-methylbenzene [FL-no: 01.010] from subgroup IVe (alkyl substituted benzene hydrocarbons), a NOAEL was available giving an adequate margin of safety compared to the estimated level of intake.

For eight substances [FL-no: 01.003, 01.004, 01.007, 01.009, 01.017, 01.024, 01.026 and 01.029] adequate margins of safety compared to the estimated levels of intake were estimated based upon a NOAEL from a 90-day study in rats, for the representative substance ß-caryophyllene [FL-no: 01.007]. The NOAEL of 222 mg/kg bw per day provides a margin of safety of 40 000 for ß-caryophyllene. The margins of safety for [FL-no: 01.003, 01.004, 01.009, 01.017, 01.024, 01.026 and 01.029] based upon the respective MSDI for these substances and the NOAEL for ß-caryophyllene, range between 7400 and $1.1x10^7$. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be evaluated at step B4 in the Procedure as being of no safety concern.

For four substances [FL-no: 01.008, 01.018, 01.040 and 01.061] adequate margins of safety compared to the estimated levels of intake were estimated based upon a NOAEL from a 90-day study in rats, for the representative substance myrcene [FL-no: 01.008]. The NOAEL of 44 mg/kg bw per day provides a margin of safety of 9100 for myrcene. The margins of safety for [FL-no 01.018, 01.040 and 01.061] based upon the respective MSDI for these substances and the NOAEL for myrcene are $4.8x10^5$, $4.4x10^6$ and $1.1x10^7$, respectively. The Panel agrees that this provides sufficient safety margins and that these flavouring substances can be evaluated at step B4 in the Procedure as being of no safety concern.

For two substances use levels have been provided by the Industry [FL-no: 01.008 and 01.026]. The mTAMDI figures calculated were above the threshold of concern for both of these substances for which more reliable exposure data are needed. On the basis of such additional data [FL-no: 01.008 and 01.026] should be reconsidered using the Procedure. For the remaining 19 substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment to finalise the evaluation.

In order to determine whether the conclusion for the 21 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications.

Adequate specifications including complete purity criteria and identity are available for all 21 JECFA evaluated substances.

Thus, for the 21 substances[15] [FL-no: 01.002, 01.003, 01.004, 01.005, 01.006, 01.007, 01.008, 01.009, 01.010, 01.016, 01.017, 01.018, 01.019, 01.020, 01.024, 01.026, 01.029, 01.040, 01.045, 01.061 and 01.077] the Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

[15] The 22nd substance, 1-methylnaphthalene [FL-no: 01.014] is in the process of being deleted from the Union List (DG SANTE, 2015).

SUMMARY OF GENOTOXICITY DATA

Table 5: Genotoxicity Data (*in vitro / in vivo*) for Aliphatic and Alicyclic Hydrocarbons (JECFA, 2006a)

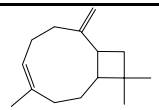
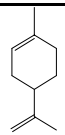
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
01.009 1323	Camphene		Reverse mutation	*S. typhimurium* TA98, TA100	0.05–100 µl/plate (42.1–84,200 µg/plate)[1]	Negative[b]	(Rockwell and Raw, 1979)
			Reverse mutation	*S. typhimurium* TA98, TA100, UTH8413, UTH8414	10–1000 µg/plate	Negative[c]	(Connor et al., 1985)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells		Negative[f]	(Sasaki et al., 1989)
					10–1000 µmol/l (1.4–136.2 µg/ml)[d,e]		
01.007 1324	β-Caryophyllene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.1–150 µl/plate (90.4–135 525 µg/plate)[g]	Negative[c]	(Jagannath, 1984a)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 150 000 µg/plate	Negative[c]	(Heck et al., 1989)
			Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535	3.3–333 µg/plate -S9[h] 1–10 000 µg/plate +S9[h]	Negative[c]	(NTP, 2004b)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells		Negative[f]	(Sasaki et al., 1989)
					10–1000 µmol/l (2.0–204.4 µg/ml)[i,e]		
			Unscheduled DNA synthesis	Rat hepatocytes	Up to 10 000 µg/ml	Negative	(Heck et al., 1989)
01.045 1326	d-Limonene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0.03–30 µmol/plate (4.1–4087 µg/plate)[k,l]	Negative[c]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0.3–33 µg/plate -S9; 10–3333 µg/plate +S9	Negative[c]	(Haworth et al., 1983; NTP, 1990)
			Reverse mutation	*S. typhimurium* TA98, TA100, UTH8413, UTH8414	10–500 µg/plate	Negative[c]	(Connor et al., 1985)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 150 000 µg/plate	Negative[c]	(Heck et al., 1989)
			Reverse mutation	*S. typhimurium* TA102	Up to 5 000 µg/plate	Negative[b]	(Müller et al., 1993)

Table 5: Genotoxicity Data (*in vitro / in vivo*) for Aliphatic and Alicyclic Hydrocarbons (JECFA, 2006a)

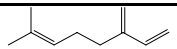
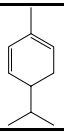
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Forward mutation, (non-)reciprocal recombination	*Saccharomyces cerevisiae MP1*	Up to 230 mmol/l (31 335 µg/ml)[k]	Negative[f]	(Fahrig, 1984)
			Forward mutation	Mouse lymphoma L5178Y Tk+/- cells	Up to 100 µg/ml	Negative[c]	(Heck et al., 1989)
			Forward mutation	Mouse lymphoma L5178Y Tk+/- cells	Up to 100 µg/ml[m]	Negative[c]	(Myhr et al., 1990; NTP, 1990)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells		Negative[f]	(Sasaki et al., 1989)
					10–333 µmol/l (1.4–45.4 µg/ml)[k,e]		
			Sister chromatid exchange	Chinese hamster ovary cells	15–162 µg/ml -S9; 16.2–162 µg/ml +S9	Negative[c]	(Anderson et al., 1990; NTP, 1990)
			Chromosomal aberration	Chinese hamster ovary cells	10–100 µg/ml -S9; 50–500 µg/ml +S9	Negative[c]	(Anderson et al., 1990; NTP, 1990)
			Cell transformation	Syrian hamster embryo cells	0.1–100 µg/ml	Negative	(Pienta, 1980)
			Cell transformation	Syrian hamster embryo cells		Positive[n]	(Rivedal et al., 2000)
					0.1–3 mmol/l (13.6–408.7 µg/ml)[k]		
01.008 1327	Myrcene		Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535	33–3 333 µg/plate -S9[o]; 33–10 000 µg/plate +S9[o]	Negative[c]	(NTP, 2004c)
			Gene mutation	Chinese hamster V79 Hprt cells	100–1 000 µg/ml	Negative[c]	(Kauderer et al., 1991)
			Sister chromatid exchange	Human lymphocytes	100–1 000 µg/ml	Negative[c]	(Kauderer et al., 1991)
			Sister chromatid exchange	Chinese hamster V79 cells	100–500 µg/ml -S9; 500 µg/ml +S9	Negative[c]	(Röscheisen et al., 1991)
			Sister chromatid exchange	Hepatic tumour cells	100–500 µg/ml	Negative[p]	(Röscheisen et al., 1991)
			Chromosomal aberration	Human lymphocytes	100–1 000 µg/ml	Negative[c]	(Kauderer et al., 1991)
01.006 1328	α-Phellandrene		Sister chromatid exchange	Chinese hamster ovary K-1 cells	33.3–1 000 µmol/l (4.5–136.2 µg/ml)[q]	Negative[f]	(Sasaki et al., 1989)

Table 5: Genotoxicity Data (*in vitro / in vivo*) for Aliphatic and Alicyclic Hydrocarbons (JECFA, 2006a)

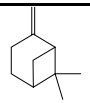
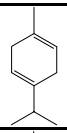
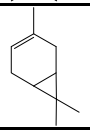
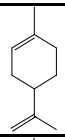
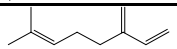
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
01.004 1329	Pin-2(3)-ene		Reverse mutation	*S. typhimurium* TA98, TA100	0.05–100 µl/plate (43–85 920 µg/plate)[r]	Negative[b]	(Rockwell and Raw, 1979)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0.03–30 µmol/plate (4.1–4 087 µg/plate)[s,l]	Negative[c]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.1–25 µl/plate (85.9–21 480 µg/plate)[r,t]	Negative[c]	(Jagannath, 1984a)
			Reverse mutation	*S. typhimurium* TA98, TA100, UTH8413, UTH8414	10–500 µg/plate	Negative[c]	(Connor et al., 1985)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 25 000 µg/plate	Negative[c]	(Heck et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	Up to 10 000 µg/ml	Negative	(Heck et al., 1989)
01.003 1330	Pin-2(10)-ene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0.03–30 µmol/plate (4.1–4 087 µg/plate)[u,v]	Negative[c]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.01–5 µl/plate (8.6–4 320 µg/plate)[w,x]	Negative[c]	(DeGraff, 1983)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 5 000 µg/plate	Negative[c]	(Heck et al., 1989)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	33.3–1 000 µmol/l (4.5–136.2 µg/ml)[u]	Negative[f]	(Sasaki et al., 1989)
01.020 1340	gamma-Terpinene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 50 000 µg/plate	Negative[c]	(Heck et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	Up to 30 µg/ml	Negative	(Heck et al., 1989)
01.029 1342	δ-3-Carene		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	1.25–5 µl/plate (2157–4 314 µg/plate)[y]	Positive[z]	(Kurttio et al., 1990)
1346	Cadinene, not in Register		Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	1–100 µg/plate -S9[1]; 100–10 000 µg/plate +S9[1]	Negative; Positive[2]	(NTP, 2004d)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	10–3 333 µg/plate -S9; 100–10 000 µg/plate +S9[o]	Negative[c]	(Haworth et al., 1983; NTP, 2004e)

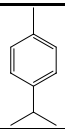
Table 5: Genotoxicity Data (*in vitro / in vivo*) for Aliphatic and Alicyclic Hydrocarbons (JECFA, 2006a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Forward mutation	Mouse lymphoma L5178Y *Tk+/- cells*	0.005–0.05 µg/ml -S9 (4.6–46.2 µg/ml)[4,5]; 0.01–0.08 µl/ml +S9 (9.2–73.9 µg/ml)[4,6]	Negative[c]	(NTP, 2004f)
			Sister chromatid exchange	Chinese hamster ovary cells	8.9–26.6 µg/ml -S9; 22.2–31.1 µg/ml +S9	Equivocal; Negative	(NTP, 2004g)
			Chromosomal aberration	Chinese hamster ovary cells	24.9–35.5 µg/ml -S9; 30.2–40 µg/ml +S9	Negative[c]	(NTP, 2004g)
In vivo							
01.045 1326	d-Limonene		Mammalian spot test	Mouse (C57BLxT) embryos	215 mg/kg bw[7]	Negative	(Fahrig, 1984)
01.008 1327	Myrcene		Chromosomal aberration	Rat bone marrow cells	100–1 000 mg/kg bw[8]	Negative[9]	(Zamith et al., 1993)
			Micronucleus formation	Mouse peripheral blood	250–2 000 mg/kg bw[10]	Negative	(NTP, 2004h)

[a] Calculated using a density of camphene of 0.842 g/ml (Lewis, 1999).
[b] With metabolic activation.
[c] With and without metabolic activation.
[d] Calculated using relative molecular mass of camphene of 136.24.
[e] Cytotoxicity observed at the highest dose/concentration tested.
[f] Without metabolic activation.
[g] Calculated using a density of β-caryophyllene of 0.9035 (0.897 - 0.910) g/ml (Lewis, 1999).
[h] Precipitation or slight toxicity was occasionally observed at the higher concentrations tested.
[i] Calculated using relative molecular mass of β-caryophyllene of 204.36.
[j] Isomer not specified.
[k] Calculated using relative molecular mass of d-limonene of 136.24.
[l] Cytotoxicity and precipitation observed at doses > 3 µmol/plate.
[m] In some trials concentrations ≥ 50 µg/ml were lethal.
[n] Although not statistically significant (p = 0.089), a fourfold increase in transformation frequency was observed.
[o] Slight toxicity was occasionally observed at the highest concentration tested.
[p] Slight increase in sister chromatid exchanges, which was reproducible but not dose-dependent.
[q] Calculated using relative molecular mass of α-phellandrene of 136.24.
[r] Calculated using a density of a-pinene of 0.8592 g/ml (Lewis, 1999).
[s] Calculated using relative molecular mass of α-pinene of 136.24.
[t] Cytotoxicity observed at doses of 2.5 to 25 µg/plate, depending on the different tester strains.

[u] Calculated using relative molecular mass of β-pinene of 136.24.
[v] Cytotoxicity observed at doses > 3 μmol/plate.
[w] Calculated using a density of β-pinene of 0.864 g/ml (Lewis, 1999).
[x] Cytotoxicity observed at doses of 2.5 to 5 μl/plate, depending on the different tester strains.
[y] Calculated using a density of d-3-carene of 0.8627 (0.8586 - 0.8668) g/ml (Merck, 1996).
[z] Positive without metabolic activation in TA100 and TA102 at doses ≥ 2.5 μl/plate; negative with metabolic activation in all strains.
[1.] Slight toxicity was observed at various doses.
[2.] Equivocal/weak positive only in TA97 and TA100 with metabolic activation.
[3.] β-Cadinene was tested.
[4.] Calculated using a density of β-cadinene of 0.9239 g/ml (Merck, 1996).
[5.] The highest concentration of 0.05 μl/ml was lethal.
[6.] In some trials, concentrations ≥ 0.04 μl/ml were lethal.
[7.] Administered via injection into the peritoneal cavity of the dam.
[8.] Administered via gavage.
[9.] A dose-related increase in mitotic index was observed, but no clastogenicity.
[10.] Administered via gavage for 90 days.

Table 6: Genotoxicity Data (*in vitro / in vivo*) for Aromatic Hydrocarbons (JECFA, 2006a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
01.002 1325	1-Isopropyl-4-methylbenzene		Reverse mutation	*S. typhimurium* TA98, TA100	0.05–100 µl/plate (42.7–85 300 µg/ plate)[a]	Negative[b]	(Rockwell and Raw, 1979)

[a] Calculated using a density of p-cymene of 0.853 g/ml (Lewis, 1999).
[b] With metabolic activation.

Table 7: Genotoxicity Data (*in vitro*) EFSA / FGE.25Rev3 (EFSA CEF Panel, in press)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
Cedrene washed[6] [CAS no 11028-42-5]	Ames test	*S. typhimurium* TA97, TA98; TA100; TA1535; TA102	8-5000[4]	Negative[1]	(Gocke, 1999)	Validity cannot be evaluated as substance is not specified. Cedarwood oil terpenes and tertonoids.
	Ames test	*S. typhimurium* TA97, TA98; TA100; TA1535; TA102	1.6-1000[5]	Negative[1]	(Gocke, 1999)	Validity cannot be evaluated as substance is not specified. Cedarwood oil terpenes and tertonoids.
Dodecane [01.038]	Ames test	*S. typhimurium* TA98; TA100	NR	Negative[1]	(Tummey et al., 1992)	Only part of abstract available. Validity of the study cannot be evaluated due to insufficient report of experimental details and results.
	Mammalian cell gene mutation test (mouse lymphoma assay)	Mouse lymphocytes	NR	Negative[1]	(Tummey et al., 1992)	Only part of abstract available. Validity of the study cannot be evaluated due to insufficient report of experimental details and results.
	Mammalian cell gene mutation test	V79 Chinese hamster ovary cells	0.12 mM (20 µg/ml)	Negative[3]	(Lankas et al., 1978)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Study designed to evaluate the ability of various alkanes to enhance the mutagenicity induced by the chemical carcinogen methylazoxymethanol acetate. Dodecane showed no mutagenic activity per se, but increased the mutagenesis induced by pretreatment with the carcinogen.
Tetradecane [01.057]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	50, 150, 500, 1500, 5000 µg/plate	Negative[1]	(PETRESA, 19??a)	(Study carried out by Huntingdon Research Centre, Report PEQ 5C/85914, sponsored by PETRESA; year not indicated) Unpublished GLP-study carried out in accordance with OECD guideline 471 as stated in the IUCLID datasheet submitted. IUCLID abstract available only. Validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	V79 Chinese hamster ovary cells	0.12 mM (23 µg/ml)	Negative[3]	(Lankas et al., 1978)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Study designed to evaluate the ability of various alkanes to enhance the mutagenicity induced by the chemical carcinogen methylazoxymethanol acetate. Tetradecane showed no mutagenic activity per se, but increased the mutagenesis induced by pretreatment with the carcinogen.

Table 7: Genotoxicity Data (*in vitro*) EFSA / FGE.25Rev3 (EFSA CEF Panel, in press)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; UTH8414; UTH8413	0, 50, 100, 500, 1000, 2000 µg/plate	Negative[1]	(Connor et al., 1985)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated. Cytotoxicity not reported.
(2-Methylbuta-1,3-diene)	Ames test	*S. typhimurium* TA98; TA100; TA1530; TA1535; TA1538	25 % atmosphere concentration	Negative[1]	(De Meester et al., 1981)	Published non-GLP study not in accordance with OECD guideline 471. Part of a larger study evaluating the effects of various experimental conditions (different liver cell preparations and concentrations) on the mutagenic activity of butadiene, hexachlorobutadiene and isoprene. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Plates were exposed to a 25 % 2-methylbuta-1,3-diene atmosphere for 24 hours.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 10000 µg/plate	Negative[1]	(Mortelmans et al., 1986) (NTP, 1999)	Published summary report including detailed results from studies on 270 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Ames test	*S. typhimurium* TA102; TA104	NR	Negative	(Kushi et al., 1985)	Published abstract only, of which part of the text including results is missing. No information on the use of a metabolic activation system. Validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535 *E. coli* WP2uvrA/pKM101	0, 500, 1000, 2000, 5000 µg/plate	Negative[1]	(Madhusree et al., 2002)	Published non-GLP study with limited report of experimental details and results. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange test	Chinese hamster ovary cells	0, 50, 160, 500, 1600 µg/ml (-S9) 0, 160, 500, 1600, 5000 µg/ml (+S9).	Negative[1]	(NTP, 1999; Galloway et al., 1987)	Published summary report including detailed results from studies on 108 chemicals tested within the NTP to a large extent in accordance with OECD guideline 479.
	Chromosomal aberration assay	Chinese hamster ovary cells	0, 1600, 3000, 5000 µg/ml	Negative[1]	(NTP, 1999; Galloway et al., 1987)	Published summary report including detailed results from studies on 108 chemicals tested within the NTP to a large extent in accordance with OECD guideline 473.
(Myrcene [01.008])	Chromosomal aberration assay	Human lymphocytes	1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Mammalian cell gene mutation assay	Chinese hamster ovary V79 cells	1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange test	Human lymphocytes	1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).

Table 7: Genotoxicity Data (*in vitro*) EFSA / FGE.25Rev3 (EFSA CEF Panel, in press)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange test	Chinese hamster ovary cells and hepatic tumour cell line	500 µg/ml	Negative[1]	(Röscheisen et al., 1991)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test (plate incorporation method)	*S. typhimurium* TA97a ; TA98; TA100; TA1535	Up to 1500 µg/plate (16 concentrations)	Negative	(Gomes-Carneiro et al., 2005)	Valid studies which were carried out with a selection of 6 of the the concentrations mentioned. In the first run concentrations up to cytotoxicity were studied; in a second run only non-toxic concentrations were tested.
	Ames	*S. typhimurium* TA97; TA98; TA100; TA1535	10 – 10 000 µg/plate	Negative[1]	(NTP, 2010)	
	Reverse mutation	*E. coli* WP2*uvrA*/pKM101	50 – 10 000 µg/plate	Negative[1]	(NTP, 2010)	
	Ames	*S. typhimurium* TA97a; TA98; TA100; TA1535	10 - 5000	Negative[1]	(Gomes-Carneiro et al., 2005)	
	Ames	*S. typhimurium* TA97a; TA98; TA100; TA1535	1 - 1500	Negative[1]	(Gomes-Carneiro et al., 2005)	
(*d*-Limonene [01.045])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/plate)	Negative[1]	(Florin et al., 1980)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 150,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA102	Up to 5000 µg/plate	Negative[1]	(Müller et al., 1993)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 3333 µg/plate	Negative[1]	(Haworth et al., 1983)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100, UTH8413 and UTH8414	0, 10 to 500 µg/plate (5 concentrations)	Negative[1]	(Connor et al., 1985)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Forward mutation assay	L5178Y Mouse lymphoma	Up to 100 µg/ml	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Forward mutation assay	L5178Y Mouse Lymphoma	Up to 100 µg/ml	Negative[1]	(Myhr et al., 1990)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Chromosomal aberration assay	Chinese hamster ovary cells	500 µg/ml	Negative[1]	(Anderson et al., 1990)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells	162 µg/ml	Negative[1]	(Anderson et al., 1990)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells	1000 µM (136.2 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(gamma-Terpinene [01.020])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	50,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Unscheduled DNA synthesis	Rat hepatocytes	30 µg/ml	Negative	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(α-Terpinene [01.019])	Ames test (plate incorporation method)	*S. typhimurium* TA97a ; TA98; TA100; TA1535	Up to 1500 µg/plate (13 concentrations)	Negative	(Gomes-Carneiro et al., 2005)	Valid studies which were carried out with a selection of 6 of the the concentrations mentioned. In the first run concentrations up to cytotoxicity were studied; in a second run only non-toxic concentrations were tested.

Table 7: Genotoxicity Data (*in vitro*) EFSA / FGE.25Rev3 (EFSA CEF Panel, in press)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
(α–Phellandrene [01.006])	Sister chromatid exchange test	Chinese hamster ovary cells	1000 µM(136.2 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(δ-3-Carene [01.029])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102	up to 5 µl/plate (up to 4300 µg/plate; 5 concentrations)	Positive[3] Negative[2]	(Kurttio et al., 1990)	Published non-GLP study with insufficiently reported results. Limited validity. Positive without metabolic activation in TA100 and TA102 and at doses of 2.5 µl/plate and higher.
(Pin-2(3)-ene [01.004])	Ames test	*S. typhimurium* TA98; TA100	100 µl/plate (85,800 µg/ plate)	Negative[2]	(Rockwell and Raw, 1979)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/ plate)	Negative[1]	(Florin et al., 1980)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	25000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	25 µl/plate (21,450 µg/ plate)	Negative[1]	(Jagannath, 1984a)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate (4290 µg/plate)	Negative[1]	(DeGraff, 1983)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; UTH8414; UTH8413	0, 10 to 500 µg/plate (5 concentrations)	Negative[1]	(Connor et al., 1985)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Unscheduled DNA synthesis	Rat hepatocytes	10000 µg/ml	Negative	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(+)-α-pinene (pin-2(3)-ene) (isomer of [01.004])	Ames test (plate incorporation method)	*S. typhimurium* TA97a ; TA98; TA100; TA1535	Up to 1000 µg/plate (18 concentrations)	Negative	(Gomes-Carneiro et al., 2005)	Valid studies.
(-)-α-pinene (pin-2(3)-ene) (isomer of [01.004])	Ames test (plate incorporation method)	*S. typhimurium* TA97a ; TA98; TA100; TA1535	Up to 4000 µg/plate (19 concentrations)	Negative	(Gomes-Carneiro et al., 2005)	Valid studies.
(Pin-2(10)-ene [01.003])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.1, 41, 410, 4100 µg/plate)	Negative[1]	(Florin et al., 1980)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange	Chinese hamster ovary cells	1000 µM (136.2 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(Camphene [01.009])	Ames test	*S. typhimurium* TA98; TA100	100 µl/plate (84,500 µg/ plate)	Negative[2]	(Rockwell and Raw, 1979)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; UTH8414; UTH8413	0, 10 to 1000 µg/plate (5 concentrations)	Negative[1]	(Connor et al., 1985)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells	1000 µM (136.2 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(β–Caryophyllene [01.007])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	150,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	150 µl/plate	Negative[1]	(Lorillard, 1984)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Longfellow, 1998)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).

Table 7: Genotoxicity Data (*in vitro*) EFSA / FGE.25Rev3 (EFSA CEF Panel, in press)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange test	Chinese hamster ovary cells	1000 µM (204.4 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).

* Supporting substances are listed in brackets.
NR: Not Reported
[1] With and without S9 metabolic activation.
[2] With metabolic activation.
[3] Without metabolic activation
[4] Plate incorporation
[5] Pre-incubation
[6] An Ames test with cedrene washed (unspecified cedrene) was also submitted, but an adequate identification of the substance studied was not possible. Therefore the study is not further discussed

Table 8: Genotoxicity Data (*in vivo*) EFSA / FGE.25Rev3 (EFSA CEF Panel, in press)

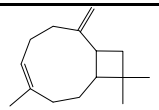
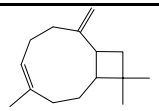
Chemical Name [FL-no]*	Test System	Test Object	Route	Dose	Result	Reference	Comments
(2-Methylbuta-1,3-diene) non-Register substance	*In vivo* Chromosomal aberration assay	Mouse (B6C3F1) bone marrow (male mice)	Inhalation	0, 438, 1750, 7000 mg/kg feed for 6 hours per day for 12 exposures over a period of 16 days (Trial 1) 0, 70, 220, 700 mg/kg feed for 6 hours per day for 12 exposures over a period of 16 days (Trial 2)	Negative	(Tice et al., 1987; Tice, 1988; Shelby, 1990)	Unpublished study report and published summary report of a valid multiple endpoint cytogenicity study sponsered by NTP, roughly in accordance with OECD guideline 475 (special dosage regimen used).
	In vivo Sister chromatid exchange test	Mouse (B6C3F1) bone marrow (male mice)	Inhalation	0, 438, 1750, 7000 mg/kg feed for 6 hours per day for 12 exposures over a period of 16 days (Trial 1) 0, 70, 220, 700 mg/kg feed for 6 hours per day for 12 exposures over a period of 16 days (Trial 2)	Positive	(Tice et al., 1987; Tice, 1988; Shelby, 1990)	Unpublished study report and published summary report of valid cytogenicity study sponsered by NTP. The study is considered valid. Significant ($0.01<p<0.05$) increase in the frequency of SCE in the bone marrow cells at all concentrations. In addition, a significant delay in bone marrow cellular proliferation kinetics (lengthening of the generation time) was detected. The mitotic index was not significantly altered.
	In vivo Micronucleus test	Mouse (B6C3F1) peripheral blood cells (male mice)	Inhalation	0, 438, 1750, 7000 mg/kg feed for 6 hours per day for 12 exposures over a period of 16 days	Positive	(Tice et al., 1987; Tice, 1988)	Unpublished study report and published summary report of valid cytogenicity study sponsored by NTP, roughly in accordance with OECD guideline 474 (special dosage regimen used). The study is considered valid. Significant ($p<0.001$) increase in the frequency of micronucleated polychromatic and normochromatic erythrocytes, and percentage of PCE. A significant ($p<0.001$) and dose-dependent decrease in the percentage of circulating polychromatic erythrocytes (suppression of erythropoiesis) was noted.
	In vivo Micronucleus test	Rat lung fibroblasts (male and female rats)	Inhalation	0, 220, 700, 7000 mg/kg feed for 13-weeks	Negative	(Khan and Heddle, 1991)	Study carried out within NTP. Only tabulated results available from NTP TR 486 (NTP, 1999). Unusual study protocol. Validity of the study cannot be evaluated.
(Myrcene [01.008])	*In vivo* Chromosomal aberration assay	Rat (Wistar) bone marrow	Gavage	0, 100, 500, 1000 mg/kg bw (single exposure)	Negative	(Zamith et al., 1993)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).

Table 8: Genotoxicity Data (*in vivo*) EFSA / FGE.25Rev3 (EFSA CEF Panel, in press)

Chemical Name [FL-no]*	Test System	Test Object	Route	Dose	Result	Reference	Comments
	In vivo Micronucleus test	Mouse (B6C3F1) peripheral blood cells	Gavage	0, 250, 500, 1000, 2000 mg/kg bw (single exposure)	Negative	(NTP, 2003)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Micronucleus assay	Mouse peripheral blood cells	Gavage	250, 500, 1000 mg/kg bw/ day	Negative	(NTP, 2010)	
(*d*-Limonene [01.045])	*In vivo* Comet assay	Mouse (ddY) / Rat (Wistar).	Oral	0, 2000 mg/kg	Negative	(Sekihashi et al., 2002)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	In vivo Mammalian spot test	Mouse embryos from C57BL/6JHan x T stocks	Intraperitoneal injection	215 mg/kg bw	Negative	(Fahrig, 1984)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	In vivo Comet assay	Rats (Sprague-Dawley) (males) (Kidneys)	Gavage	0, 1000, 2000 mg/kg bw (single exposure)	Negative	(Nesslany et al., 2007)	
	In vivo transgenic mutagenisity assay	Rats (Big blue) (males) (liver, kidney, bladder)	Diet	0, 525 mg/kg bw per day (10 days)	Negative	(Turner et al., 2001)	The author do not specify whether the tested compound is *d*- or *l*-limonene, and the purity of the compound is not stated. However, the stability of the limonene in the diet was measured.
Naphthalene [01.053]	*In vivo* Unscheduled DNA synthesis	Rat hepatocytes	Gavage	0, 600, 1000, 1600 mg/kg bw	Negative	(Research Toxicology Center, 1999)	Summarised report of unpublished study carried out in accordance with OECD guideline 486. Although some minor details of the results are not reported (viability of cells, individual slide values for nuclear grains and cytoplasmic grains) the study is considered valid.
	In vivo Micronucleus test	Mouse (Swiss ICR) bone marrow	Gavage	50, 250, 500 mg/kg bw (single exposure)	Negative	(Harper et al., 1984)	Published non-GLP study not fully in accordance with OECD guideline 474 (only males tested, sampling time not indicated, effect on PCE/NCE ratio not reported). Due to the limited report of experimental details and results the validity of the study cannot be evaluated. At the dose of 500 mg/kg bw two of ten animals died. The dose of 1500 mg/kg bw was toxic (lethal) to all animals. Induction of micronuclei in benzene-treated mice was significantly enhanced by co-treatment with naphthalene at 50 and 250 mg/kg bw.
	In vivo Micronucleus test	Mouse (CD-1) bone marrow	Intraperitoneal injection	250 mg/kg bw (single exposure)	Negative	(Sorg et al., 1985)	Unpublished valid GLP-study carried in accordance with OECD guideline 474. Naphthalene was negative in the micronucleus test at the dose of 250 mg/kg bw at all of the time intervals tested. A harvest-time dependent depression in the PCE/NCE ratio was observed in animals treated with the test substance, which was statistically significant ($p \leq 0.05$) at sacrifice time of 72 hours.

* Supporting substances are listed in brackets.

Table 9: Genotoxicity Data of β-Caryphyllene Submitted by EFFA (EFFA, 2012)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference	Comments
In vitro								
01.007 1324	β-Caryophyllene		Reverse mutation	*S. typhimurium* TA98, TA100, *E. coli* WP2uvrA	2300–9000 µg/plate	Negative[1]	(Di Sotto et al., 2008)	Study not in compliance with OECD 471: - assay does not include TA1535 and TA1537 (or TA97, 97a, resp) -5 concentrations tested (plate-incorpration) but not given in detail (authors stated that in range finder up to 9 mg/plate without cytotoxicity) -results not given in detail (no values, no raw data). Methods and results poorly reported -no historical contral data insufficient quality.
In vivo								
01.007 1324	β-Caryophyllene		Micronucleated polychromatic erythrocytes	Mice	0, 20, 200 and 2000 mg/kg bw[2]	Negative	(Molina-Jasso et al., 2009)	In compliance with OECD 474 -except: number of micronucleated immature erythrocytes not given separately for each animal; historical controls not given. Reliable with restrictions=limited validity.

SUMMARY OF TOXICITY DATA

Table 10: Toxicity Data Considered by the Panel in FGE.78Rev2

Chemical Name [FL-no:]	Species; Sex No/group	Route	Doses (mg/kg bw per day)	Duration (days)	NOAEL (mg/kg bw per day)	Reference	Comments
(Myrcene [01.008])	Rat; M, F 4/10	Diet	0, 8.0, 40 and 44 mg/kg bw per day (males) 0, 9.6, 48 and 53 mg/kg bw per day (females)	90 days	44 (males) 53 (females)	(Bauter, 2013b)	No toxicity at highest dose level.
(β-Caryophyllene [01.007])	Rat; M, F 4/20	Diet	0, 222, 456 and 1.367 mg/kg bw per day (males) 0, 263, 1.033 and 4.278 mg/kg bw per day (females),	3 months	222 (males) 263(females)	(Bauter, 2013a)	Study according to OECD Guideline 408.
	Rat; M, F 4/6	Diet	0, 516, 1.547 and 3.569 mg/kg bw per day (males) 0, 528, 1.582 and 4.438 mg/kg bw per day (females)	14 days	-	(Bauter, 2011)	

SUMMARY OF SAFETY EVALUATIONS

Table 11: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

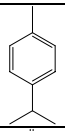
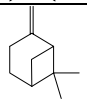
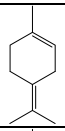
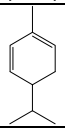
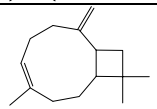
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (μg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
01.002 1325	1-Isopropyl-4-methylbenzene		926 472	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.003 1330	Pin-2(10)-ene		1300 759	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.005 1331	Terpinolene		660 70	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.006 1328	alpha-Phellandrene		79 410	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.007 1324	beta-Caryophyllene		330 508	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.009 1323	Camphene		13 28	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 11: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

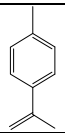
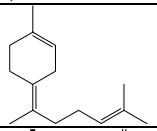
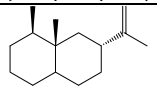
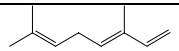
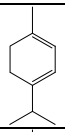
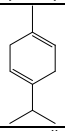
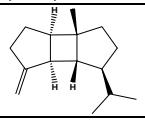
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
01.010 1333	1-Isopropenyl-4-methylbenzene		18 0.3	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.016 1336	1,4(8),12-Bisabolatriene		13 10	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.017 1337	Valencene		53 26	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.018 1338	beta-Ocimene		55 11	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.019 1339	alpha-Terpinene		28 93	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.020 1340	gamma-Terpinene		1200 321	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.024 1345	beta-Bourbonene		0.012 0.2	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 11: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

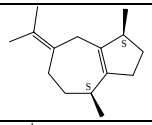
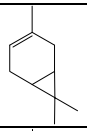
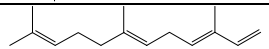
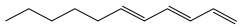
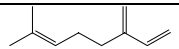
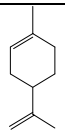
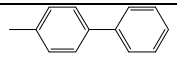
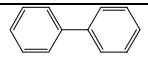
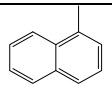
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
01.026 1347	1(5),7(11)-Guaiadiene		0.012 3	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.029 1342	delta-3-Carene		290 40	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.040 1343	alpha-Farnesene		0.61 40	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.061 1341	Undeca-1,3,5-triene		0.24 0.2	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.077 1344	1-Methyl-1,3-cyclohexadiene		0.012 313	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.004 1329	Pin-2(3)-ene		1800 2444	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 11: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
01.008 1327	Myrcene		290 153	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.045 1326	d-Limonene		34000 12726	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
01.011 1334	4-Methyl-1,1'-biphenyl		0.0085 0.08	Class III A3: Intake below threshold	d	Genotoxicity data required.	No longer supported by Industry (DG SANCO, 2012).
01.013 1332	Biphenyl		0.00085 0.7	Class III A3: Intake below threshold	d	Genotoxicity data required.	No longer supported by Industry (DG SANCO, 2012).
01.014 1335	1-Methylnaphthalene		0.73 0.06	Class III A3: Intake below threshold	d	The Commission has communicated that this substance is in the process of being deleted from the Union List (October 2014).	The Commission has communicated that this substance is in the process of being deleted from the Union List (October 2014).

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

Table 12: Summary of Safety Evaluation by the EFSA (FGE.25Rev3) (EFSA CEF Panel, in press)

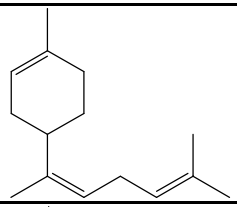
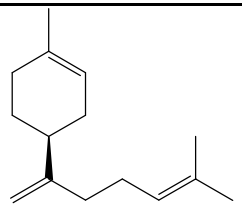
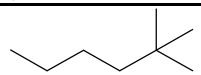
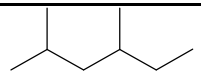
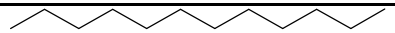
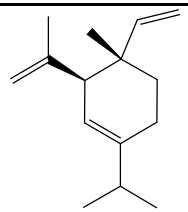
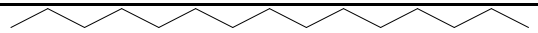
FL-no	EU Register name	Structural formula	EU MSDI [a] (µg/*capita* per day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f],[g], or [h]]	Evaluation remarks
01.027	Bisabola-1,8,12-triene		0.024	Class I A3: Intake below threshold	d	g	
01.028	beta-Bisabolene		2.7	Class I A3: Intake below threshold	d	f	
01.033	2,2-Dimethylhexane		1.2	Class I A3: Intake below threshold	d	f	
01.034	2,4-Dimethylhexane		1.2	Class I A3: Intake below threshold	d	f	
01.038	Dodecane		0.012	Class I A3: Intake below threshold	d	f	
01.039	delta-Elemene		0.012	Class I A3: Intake below threshold	d	f	
01.054	Pentadecane		0.61	Class I A3: Intake below threshold	d	f	

Table 12: Summary of Safety Evaluation by the EFSA (FGE.25Rev3) (EFSA CEF Panel, in press)

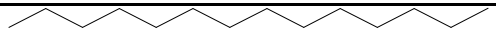
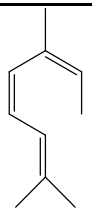
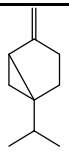
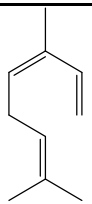
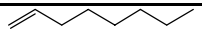
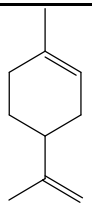
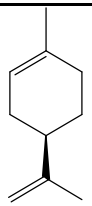
FL-no	EU Register name	Structural formula	EU MSDI [a] (µg/*capita* per day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f],[g], or [h]]	Evaluation remarks
01.057	Tetradecane		0.012	Class I A3: Intake below threshold	d	f	
01.035	2,6-Dimethylocta-2,4,6-triene		9.1	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.059	4(10)-Thujene		14	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	d		
01.064	cis-3,7-Dimethyl-1,3,6-octatriene		14	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.070	1-Octene		0.0085	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.001	Limonene		4000	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	f	

Table 12: Summary of Safety Evaluation by the EFSA (FGE.25Rev3) (EFSA CEF Panel, in press)

FL-no	EU Register name	Structural formula	EU MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)],[(g)], or [(h)]]	Evaluation remarks
01.046	l-Limonene		2100	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	f	

(a): EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita per day.
(b): Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): 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.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.

DOCUMENTATION PROVIDED TO EFSA

1. Bauter MR, 2011. β-Caryophyllene: palatability/toxicity study: a 14-day dietary study in rats. Product Safety Labs. Study no. 31085. November 16, 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

2. Bauter MR, 2013a. β-Caryophyllene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33328. January 7, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

3. Bauter MR, 2013b. Myrcene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33546. May 20, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

4. DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/02 2012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.

5. DG SANTE (Directorate General for Health and Food safety), 2015. Information from DG SANTE 04 March 2015, concerning the status of substance [FL-no: 01.014] in FGE.78.

6. Di Sotto A, Evandri MG and Mazzanti G, 2008. Antimutagenic and mutagenic activities of some terpenes in the bacterial reverse mutation assay. Mutation Research 653, 130-133.

7. EFFA (European Flavour and Fragrance Association), 2005. Submission 2004-3. Flavouring group evaluation of 32 flavouring substances (candidate chemicals) of chemical group 31 (annex I of 1565/2000/EC) structurally related to aliphatic and aromatic hydrocarbons [FEMA 2004-2] used as flavouring substances. 24 June 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.38.

8. EFFA (European Flavour Association), 2012. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 31 (Annex I of 1565/2000/EC) Aliphatic and Alicyclic and Aromatic Hydrocarbons [JECFA/WHO FAS 54] Structurally Related to Aliphatic and Aromatic Hydrocarbons as Evaluated by EFSA in FGE.25Rev2 and FGE.78Rev1. Addendum to FGE.25Rev2 and FGE.78Rev1. December 2012. FLAVIS/8.176.

9. EFFA (European Flavour Association), 2013. E-mail from EFFA to EFSA and FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 30 April 2013. Information on specifications and stereoisomeric/ positional composition of substances evaluated in FGE.78Rev2: [FL-no: 01.004, 01.007, 01.008, 01.009, 01.017, 01.018, 01.019, 01.020, 01.024, 01.026, 01.029, 01.040, 01.045, 01.061]. FLAVIS/8.191.

10. EFFA (European Flavour Association), 2014. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 13 January 2014. Information on substances in FGE.74Rev3 [FL-no: 12.238, 12.239 and 12.291], FGE.78Rev2 [FL-no: 01.004, 01.018, 01.019, 01.040, 01.045 and 01.061] and FGE.91Rev2 [FL-no: 12.038 and 12.085]. FLAVIS/8.219.

11. EFFA (European Flavour Association), 2015. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 13 January 2015. Information on substance [FL-no: 01.004] in FGE.78Rev2.

12. Flavour Industry, 2010. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-78Rev1/A-25Rev2 [FL-no: 01.008, 01.022, 01.035, 01.047, 01.064].

13. Molina-Jasso D, Álvarez-Gonzáles I and Madrigal-Bujaidar E, 2009. Clastogenicity of β-caryophyllene in mouse. Biological and Pharmaceutical Bulletin 32(3), 520-522.

14. Zook C and Garlick D, 2013. β-Caryophyllene: a histopathological assessment of the male rat kidney. Histo-Scientific Research Laboratories. Product Safety Labs. Study no. 36206. July 30, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

REFERENCES

Anderson BE, Zeiger E, Shelby MD, Resnick MA, Gulati DK, Ivett JL and Loveday KS, 1990. Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environmental and Molecular Mutagenesis 16(Suppl. 18), 55-137.

Bagchi D, Balmoori J and Bagchi M, 2000. Role of p53 tumor suppressor gene in the toxicity of TCDD, endrin, naphthalene, and chromium (VI) in liver and brain tissues of mice. Free Radical Biology and Medicine 28(6), 895-903.

Barfknecht TR, Naismith RW and Matthews RJ, 1985. Rat hepatocyte primary culture/DNA repair test using test article 5601-56-1 (naphthalene). Texaco Inc. EPA Doc 86-870000560, microfiche no. OTS0513638. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2011. β-Caryophyllene: palatability/toxicity study: a 14-day dietary study in rats. Product Safety Labs. Study no. 31085. November 16, 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2013a. β-Caryophyllene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33328. January 7, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2013b. Myrcene: a 90-day dietary study in rats. Product Safety Labs. Study no. 33546. May 20, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Clark CR, Sanchez A and Hobbs CH, 1977. Toxicology of solar heating and cooling materials: mutagenic survey of heat transfer fluids. In: Boecker, B.B., Hobbs, C.H., Martinez, B.S. (eds). Annual report of the Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Inc., Albuquerque, New Mexico, USA. Unpublished report. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States.

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

Connor TH, Theiss JC, Hanna HA, Monteith DK and Matney TS, 1985. Genotoxicity of organic chemicals frequently found in the air of mobile homes. Toxicology Letters 25, 33-40.

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

De Meester C, Mercier M and Poncelet F, 1981. Mutagenic activity of butadiene, hexachlorobutadiene, and isoprene. Industrial and Environmental Xenobiotics, Proceedings of an International Conference. 27-30 May 1980, 195-203.

De Rijk EPCT, Ravesloot WTM, Wijnands Y and Van Esch E, 2003. A fast histochemical staining method to identify hyaline droplets in the rat kidney. Toxicologic Pathology 31(4), 462–4.

Dean BJ, 1980. Toxicity studies with detergent intermediates: *In vitro* genotoxicity studies with SHOP process components. Sittingbourne, Shell Research Ltd. TLGR.80.074, October 1980. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 112-41-4, EINECS Name dodec-1-ene. Section 1.0.1-5.11.

DeGraff WG, 1983. Mutagenicity evaluation of β-pinene in the Ames salmonella/microsome plate test. Final report. Litton Bionetics, Inc. LBI project no. 20988. October, 1983. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Delgado-Rodriques A, Ortiz-Marttelo R and Graf U, 1995. Genotoxic activity of environmentally important polycyclic aromatic hydrocarbons and their nitro derivates in the wing spot test of *Drosophila melanogaster*. Mutation Research 341, 235-247.

Di Sotto A, Evandri MG and Mazzanti G, 2008. Antimutagenic and mutagenic activities of some terpenes in the bacterial reverse mutation assay. Mutation Research 653, 130-133.

Djomo JE, Ferrier V and Gauthier L, 1995. Amphibian micronucleus test *in vivo*: Evaluation of the genotoxicity of some major polycyclic aromatic hydrocarbons found in a crude oil. Mutagenesis 10, 223-226.

EC (European Commission), 2000. 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.

ECB (European Chemical Bureau), 2005. Classification & Labelling. Annex I of Directive 67-548-EEC. Available at: www.ecb.jrc.it/classification-labelling/.

EFSA (European Food Safety Authority), 2004. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food (AFC) on a request from the Commission related to Flavouring Group Evaluation 03 (FGE.03): Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes, and an orthoester of formic acid, from chemical groups 1 and 2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal 2004, 107, 1-59.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food (AFC) related to Flavouring Group Evaluation 78: Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal (2009) 931, 1-59.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011a. Scientific Opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1): Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2. EFSA Journal 2011;9(6):2178, 69 pp. doi:10.2903/j.efsa.2011.2178

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011b. Scientific Opinion on Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2): Aliphatic and aromatic hydrocarbons from chemical group 31. EFSA Journal 2011; 9(6):2177, 126 pp. doi:10.2903/j.efsa.2011.2177

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012. Scientific Opinion. Statement on List of Representative Substances for Testing. EFSA Journal 2012;10(3):2639, 9 pp. doi:10.2903/j.efsa.2012.2639

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), in press. Scientific Opinion on Flavouring Group Evaluation 25, Revision 3 (FGE.25Rev3): Aliphatic and aromatic hydrocarbons from chemical group 31. Not published yet.

EU-RAR (European Union Risk Assessment Report), 2003. EU-RAR on naphthalene (CAS no: 91-20-3; EINECS no: 202-049-5). Final Report 2003. European Chemicals Bureau, Institute for Health and Consumer Protection, Ispra, Italy.

Fahrig R, 1984. Genetic mode of action of carcinogens and tumor promoters in yeast and mice. Molecular and General Genetics 194, 7-14.

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.

Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B and Bube A, 2012. Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicologic Pathology 40, 14s-86s.

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, 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environmental and Molecular Mutagenesis 10(Suppl. 10), 1-175.

Gocke E, 1999. Mutagenicity evaluation of cedrene washed in the Ames Test. Roche LtD. Project No. 489M99. November 16, 1999. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Godek EG, Naismith RW and Matthews RJ, 1985. Ames Salmonella/microsome plate test (EPA/OECD) using test article 5601-56-1 (naphthalene). Texaco Inc. EPA Doc 86-870000559, microfiche no. OTS0513637. Date 3/26/85. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Gollahon LS, 1991. Chromosomal damage to preimplantation mouse embryos *in vitro* by naphthalene and aflatoxin B1. Dissertation Abstracts International 52, 694-B.

Gomes-Carneiro MR, Viana MES, Felzenszwalb I and Paumgartten FJR, 2005. Evaluation of β-myrcene, α-terpinene and (+)- and (-)-α-pinene in the salmonella/microsome assay. Food and Chemical Toxicology 43, 247-252.

Hard GC, Rodgers IS, Baetchke KP, Richards WL, McGaughy RE and Valcovic LR, 1993. Hazard evaluation of chemicals that cause accumulation of α2u-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. Environmental Health Perspectives 99, 313-349.

Harper BL, Sadagopa Ramanujam VM, Gad-El-Karim MM and Legator MS, 1984. The influence of simple aromatics on benzene clastogenicity. Mutation Research 128, 105-114.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environmental Mutagenesis 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.

IARC (International Agency for Research on Cancer), 1999. Summaries and evaluations, isoprene (group 2b), vol. 71, part three, p. 1015. International Programme on Chemical Safety, International Agency for Research on Cancer, WHO, Lyon.

Jagannath DR, 1984a. Mutagenicity evaluation of α-pinene in the Ames salmonella/microsome plate test. Final report. Litton Bionetics Inc. LBI project no. 20988. January, 1984. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Jagannath DR, 1984b. Mutagenicity evaluation of B158 in the Ames Salmonella/microsome plate test (b-caryophyllene). Unpublished report No. 7195 from Litton Bionetics, Inc., Kensington, Maryland, USA. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States.

Japan Chemical Industry (Date not given) Ecology - Toxicology and Information Center. Mutagenicity of test data of existing chemical substances based on the toxicity investigation of the Industrial Safety and Health Law. Naphthalene. Carcinogenicity studies. Mutagenicity studies. Cited in CCRIS.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 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 (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. 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 (Joint FAO/WHO Expert Committee on Food Additives), 1999. 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 (Joint FAO/WHO Expert Committee on Food Additives), 2005a. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005b. 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 (Joint FAO/WHO Expert Committee on Food Additives), 2006a. Safety evaluation of certain food additives and contaminants. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.

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

Kaden DA, Hites RA and Thilly WG, 1979. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. Cancer Research 39, 4152-4159.

Kauderer B, Zamith H, Paumgartten JR and Speit G, 1991. Evaluation of the mutagenicity of β-myrcene in mammalian cells *in vitro*. Environmental and Molecular Mutagenesis 18, 28-34.

Khan MA and Heddle JA, 1991. Chemical induction of somatic gene mutations and chromosomal aberrations in lung fibroblasts of rats. Mutation Research 263(4), 257-262.

Kulka U, Schmid E, Huber R and Bauchinger M, 1988. Analysis of the cytogenetic effect in human lymphocytes induced by metabolically activated 1and 2-methylnaphthalene. Mutation Research 208(3/4), 155-158.

Kurttio P, Kalliokoske P, Lampelo S and Jantunen MJ, 1990. Mutagenic compounds in wood-chip drying fumes. Mutation Research 242, 9-15.

Kushi A, Yoshida D and Mizusaki S, 1985. Mutagenicity of gaseous nitrogen oxides and olefins on Salmonella TA102 and TA104. Mutation Research 147(5), 263-264.

Lankas GR, Baxter CS and Christian RT, 1978. Effect of alkane tumor-promoting agents on chemically induced mutagenesis in cultured V79 Chinese hamster cells. Journal of Toxicology and Environmental Health 4, 37-41.

Lawlor TE, 1994. Letter from first chem. corp. to USEPA re. mutagenicity test on NRS 1020 and NRS 10W40 in the Salmonella/mammalian-microsome reverse mutation assay (Ames test) with attachments. Naphthalene. First Chemical Corporation. EPA Doc 86940001856, microfiche no. OTS0558051. Date 8/8/94. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Longfellow D, 1991. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute. Naphthalene. Carcinogenicity studies. Mutagenicity studies. Cited in CCRIS.

Longfellow D, 1998. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute. Beta-caryophyllene. Mutagenicity studies. Cited in CCRIS.

Lorillard (Lorillard Tobacco Company), 1984. Mutagenicity evaluation of β-caryophyllene in the Ames Salmonella/microsome plate test. Final report. Litton Bionetics, Inc. LBI Project no. 20988. January, 1984. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Madhusree B, Goto S, Ohkubo T, Tian H, Ando F, Fukuhara M, Tohkin M and Watanabe I, 2002. Mutagenicity testing of 1,3-butadiene, 1,4-pentdiene-3-ol, isoprene, 2,4-hexadiene, cis- and trans-piperlylene. Journal of Health Science 48(1), 73-78.

Mamber SW, Bryson V and Katz SE, 1984. Evaluation of the *Escherichia coli* K12 inductest for detection of potential chemical carcinogens. Mutation Research 130, 141-151.

McCann J, Choi E, Yamasaki E and Ames BN, 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proceedings of the National Academy of Sciences of the United States of America 72(12), 5135-5139.

Molina-Jasso D, Álvarez-Gonzáles I and Madrigal-Bujaidar E, 2009. Clastogenicity of β-caryophyllene in mouse. Biological and Pharmaceutical Bulletin 32(3), 520-522.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E, 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. Environmental and Molecular Mutagenesis 8(Suppl. 7), 1-119.

Myhr B, McGregor D, Bowers L, Riach C, Brown AG, Edwards I, McBride D, Martin R and Caspary WJ, 1990. L5178Y mouse lymphoma cell mutation assay results with 41 compounds. Environmental and Molecular Mutagenesis 16(Suppl. 18), 138-167.

Müller W, Engelhart G, Herbold B, Jäckh R and Jung R, 1993. Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories. Environmental Health Perspectives (Suppl. 101(3)), 33-36.

Nesslany F, Zennouche N, Simar-Meintieres S, Talahari I, NKili-Mboui E-N and Marzin D, 2007. In vivo Comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds. Mutation Research 630, 28-41.

NTP (National Toxicology Program), 1990. NTP technical report on the toxicology and carcinogenesis studies of d-limonene (CAS no. 5989-27-5) in F344/N rats and B6C3F1 mice (gavage studies). January 1990. NTP-TR 347. NIH Publication no. 90-2802.

NTP (National Toxicology Program), 1992. Toxicology and carcinogenesis studies of naphthalene (CAS no. 91-20-3) in B6C3F1 mice (inhalation studies). April 1992. NTP-TR- 410. NIH Publication no. 92-3141.

NTP (National Toxicology Program), 1999. Toxicology and carcinogenesis studies of isoprene (CAS no. 78-79-5) in F344/N rats and B6C3F1 mice (inhalation studies). NIH Publication no.99-3976. NTP-TR 486. 1-176.

NTP (National Toxicology Program), 2003. Initial study results from a 90-day toxicity study on β-myrcene in mice. Study number C99023.

NTP (National Toxicology Program), 2004a. Draft study results of a 13-week gavage study in rats (β-myrcene). Study no. C099023. [Online].

NTP (National Toxicology Program), 2004b. Salmonella study results (β-caryophyllene). Study no. 663295. [Online].

NTP (National Toxicology Program), 2004c. Salmonella study results (β-myrcene). Study no. A96914. [Online].

NTP (National Toxicology Program), 2004d. Salmonella study results (cadinene). Study no. 385918. [Online].

NTP (National Toxicology Program), 2004e. Salmonella study results (β-cadinene). Study no. 519172. [Online].

NTP (National Toxicology Program), 2004f. Mouse lymphoma study results (β-cadinene). Study no. 138289. [Online].

NTP (National Toxicology Program), 2004g. Sister chromatid exchange and chromosome aberration study results (β-cadinene). Study no. 972780. [Online].

NTP (National Toxicology Program), 2004h. Study results of micronucleus study in peripheral blood of mice (β-myrcene). Study no. A06528. [Online].

NTP (National Toxicology Program), 2010. Toxicology and carcinogenesis studies of β-myrcene (CAS No. 123-35-3) in F344/N rats and B6C3F1 mice (gavage studies). National Toxicology Program, Research Triangle, NC, USA. TR-557. NIH Publication No. 08-5898. [Online] http://ntp.niehs.nih.gov/.

PETRESA (date not given) Huntingdon Research Centre Report N° 84450D/PEQ/1/AC. Cited in European Commission - European Chemicals Bureau, 2000. IUCLID Dataset, Substance ID: 629-59-4, EINECS Name tetradecane. Section 1.0.1-5.11.

Pienta RJ, 1980. Evaluation and relevance of the Syrian hamster embryo cell system. In: Williams, G.M. (Ed.) The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation. Elsevier/North-Holland Biomedical Press, pp. 149-169.

Posternak NM, Linder A and Vodoz CA, 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. Food and Cosmetics Toxicology 7, 405-407.

Research Toxicology Center, 1999. Naphthalene unscheduled DNA synthesis (UDS) after in vivo treatment. Monitored by Rutgers VFT AG; sponsored by International Tar Assoc. Research Toxicology Center, Rome. Cited in Schreiner, C.A., 2003. Genetic toxicity of naphthalene: a review. Journal of Toxicology and Environmental Health, Part B 6(2), 161-183.

Rivedal E, Mikalsen SO and Sanner T, 2000. Morphological transformation and effect on gap junction intercellular communication in Syrian hamster embryo cells as screening tests for carcinogens devoid of mutagenic activity. Toxicology In Vitro 14, 185-192.

Rockwell P and Raw I, 1979. A mutagenic screening of various herbs, spices and food additives. Nutrition and Cancer 1(4), 10-15.

Roscheisen C, Zamith H, Paumgartten F and Speit G, 1991. Influence of β-myrcene on sister chromatid exchanges induced by mutagens in V79 and HTC cells. Mutation Research 264, 43-49.

Sakai M, Yoshida D and Mizusaki S, 1985. Mutagenicity of polycyclic aromatic hydrocarbons and quinines on *Salmonella typhimurium* TA97. Mutation Research 156, 61-67.

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. Mutation Research 226, 103-110.

Sasaki JC, Arey J, Eastmond DA, Parks KK and Grosovsky AJ, 1997. Genotoxicity induced in human lymphoblasts by atmospheric reaction products of naphthalene and phenanthrene. Mutation Research 393(1-2), 23-35.

SCF (Scientific Committee for Food), 1999. 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 to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, Knasmüller S, Tsuda S and Sasaki YF, 2002. Comparative investigation of multiple organs of mice and rats in the comet assay. Mutation Research 517(1-2), 53-75.

Shelby MD, 1990. Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. Environmental Health Perspectives 86, 71-73.

Sina JF, Bean CL, Dysart GR, Taylor VI and Bradley MO, 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutation Research 113, 357-391.

Sokolowski A, 2001. Salmonella typhimurium reverse mutation assay with longifolene (CN). Project no. 697103. RCC Cryotest Cell Research GMBH, Integen, Germany. Unpublished report to the Research Institute for Fragrance Materials, Woodcliff Lake, NJ, USA.

Sorg RM, Naismith RW and Matthews RJ, 1985. Micronucleus test using test article 5601-56-1. Naphthalene. Texaco Inc. EPA Doc 86-870000561, microfiche no. OTS0513639. Date 10/31/85. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Stankowski LF, 1987. Ames/Salmonella plate incorporation assay using test article 5601-56-1 with cover letter dated 12/17/87. Texaco Inc. EPA Doc 86-880000090, microfiche no. OTS0513979. Date 11/25/87. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Tice RR, Boucher R, Luke CA, Paquette DE, Shelby MD and Melnick RL, 1987. Chloroprene and isoprene: Cytogenetic studies in mice with cover letter dated 07/30/87. U.S. EPA. EPA Doc 8EHQ-0887-0689, microfiche no. OTS0513405. Date 8/24/87. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Tice RR, 1988. The cytogenetic evaluation of in vivo genotoxic and cytotoxic activity using rodent somatic cells. Cell Biology and Toxicology 4(4), 475-486.

Tingle MD, Pirmohamed M, Templeton E, Wilson AS, Madden S, Kitteringham NR and Park BK, 1993. An investigation of the formation of cytotoxic, genotoxic, protein-reactive and stable metabolites from napthalene by human liver microsomes. Biochemical Pharmacology 46, 1529-1538.

Tummey AC, McKee RH and Przygoda RT, 1992. Evaluation of *in-vivo* tumor promoters in a battery of in-vitro assays. Environmental and Molecular Mutagenesis 19(Suppl. 20), 66.

Turner SD, Tinwell H, Piegorsch W, Schmezer P and Ashby J, 2001. The male rat carcinogens limonene and sodium saccharin are not mutagenic to male Big Blue rats. Mutagenesis 16(4), 329-332.

US ATSDR, 2005. Toxicological profile for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. August, 2005. Accessible via: http:/www.atsdr.cdc.gov

WHO, 1998. Environmental Health Criteria 202. Selected non-heterocyclic polycyclic aromatic hydrocarbons. International Programme on Chemical Safety, WHO, Geneva.

Wilson AS, Tingle MD, Kelly MD and Park BK, 1995. Evaluation of the generation of genotoxic and cytotoxic metabolites of benzo(a)pyrene, aflatoxin B, naphthalene and tamoxifen using human liver microsomes and human lymphocytes. Human and Experimental Toxicology 14, 507-515.

Wilson AS, Davis CD, Williams DP, Buckpitt AR, Pirmohamed M and Park BK, 1996. Characterization of the toxic metabolite(s) of naphthalene. Toxicology 114, 233-242.

Zamith HP, Vidal MNP, Speit G and Paumgartten FJR, 1993. Absence of genotoxic activity of β-myrcene in the *in vivo* cytogenetic bone marrow assay. Brazilian Journal of Medical and Biological Research 26, 93-98.

Zook C and Garlick D, 2013. β-Caryophyllene: a histopathological assessment of the male rat kidney. Histo-Scientific Research Laboratories. Product Safety Labs. Study no. 36206. July 30, 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

ABBREVIATIONS

BMDL	Benchmark dose level
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFFA	European Flavour and Fragrance Association
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
GC-MS	Gas chromatography-mass spectrometry
ID	Identity
I.p.	Intraperitoneal
IR	Infrared spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
MOS	margin of safety
NCE	Normochromatic erythrocyte
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development

PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
WHO	World Health Organization