EFSA 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

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SCIENTIFIC OPINION

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[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 24 aliphatic, alicyclic and aromatic hydrocarbons evaluated by the JECFA (65th meeting). In the previous version of 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) have now become available for beta-myrcene [FL-no: 01.008] and the present revision of FGE.78, FGE.78Rev1, includes the evaluation of these data. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. Two substances [FL-no: 01.011 and 01.013] are genotoxic *in vitro* and potentially carcinogenic, and are therefore not evaluated using the Procedure. The Panel concluded that the nine substance [FL-no: 01.002, 01.005, 01.006, 01.010, 01.016, 01.019, 01.020, 01.045 and 01.077] do not give rise to safety concerns at the levels of dietary intake, estimated on the basis of the MSDI approach. For 13 substances [FL-no: 01.003, 01.004, 01.007, 01.008, 01.009, 01.014, 01.017, 01.018, 01.024, 01.026, 01.029, 01.040 and 01.061] additional toxicity data are requested. For one substance [FL-no: 01.024] EU production

1 On request from the Commission, Question No EFSA-Q-2010-01557, adopted on 19 May 2011.

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

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figure is needed to finalise the evaluation. Besides the safety assessment of these substances, the specifications for the materials of commerce have been considered. For two substances [FL-no: 01.018 and 01.061] the isomeric composition is lacking. 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] further information on the composition of mixture is requested.

SUMMARY

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

The present consideration concerns 24 aliphatic, alicyclic and aromatic hydrocarbons evaluated by the JECFA (65th meeting). The Panel concluded that the 24 substances are structurally related to the group of 37 aliphatic and aromatic hydrocarbons evaluated by EFSA in the Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2).

The Panel agrees with the application of the Procedure as performed by the JECFA for eight of the 24 substances considered in this FGE [FL-no: 01.002, 01.005, 01.006, 01.016, 01.019, 01.020, 01.045 and 01.077].

Two substances [FL-no: 01.011 and 01.013] are genotoxic *in vitro* and there are unresolved problems with potential carcinogenicity and therefore the Panel concluded that they cannot be evaluated using the Procedure.

For the following 14 substances [FL-no: 01.003, 01.004, 01.007, 01.008, 01.009, 01.010, 01.014, 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 13 substances no applicable NOAEL is available for the substance itself or on a structurally related compound and therefore further data are required.

For one substance [FL-no: 01.024] the JECFA evaluation is only based on MSDI values derived from production figure from the USA. EU production figure is needed in order to finalise the evaluation of this substance.

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 structural class I to which the two substances belong. For these two substances 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 22 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 24 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 10 of the 24 JECFA evaluated substances. For two substances [FL-no: 01.018 and 01.061] information on the isomeric composition is lacking and 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] further information on the composition of the mixture is requested.

Thus, 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] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing information on stereoisomerism/composition of mixture). For two of the 24 JECFA evaluated substances the Procedure could not be applied [FL-no: 01.011 and 01.013] due to concern with respect to genotoxicity and carcinogenicity. For 13 of the 24 JECFA evaluated aliphatic, alicyclic and aromatic hydrocarbons [FL-no: 01.003, 01.004, 01.007, 01.008, 01.009, 01.014, 01.017, 01.018, 01.024, 01.026, 01.029, 01.040 and 01.061] additional toxicity data are requested. For the remaining six substances [FL-no: 01.002, 01.005, 01.006, 01.010, 01.016 and 01.077] the Panel agrees with JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

KEY WORDS

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

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BACKGROUND

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

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

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

In the period 2000 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

TERMS OF REFERENCE

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

In addition, in letter of 10 February 2010 the Commission requested EFSA to carry out a re-evaluation of flavouring substance myrcene [FL-no: 01.008] based on additional toxicity data:

"The European Commission requests the European Food Safety Authority to carry out a safety assessment on β-myrcene [FL-no: 01.008], α-cedrene [FL-no: 01.022], 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], longifolene [FL-no: 01.047] and cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] in accordance with Commission Regulation (EC) No 1565/2000, by end 2010".

The deadline of the Terms of Reference was negotiated to end 2014.

α-Cedrene [FL-no: 01.022], 2,6-dimethylocta-2,4,6-triene [FL-no: 01.035], longifolene [FL-no: 01.047] and cis-3,7-dimethyl-1,3,6-octatriene [FL-no: 01.064] were re-evaluated in FGE.25Rev2.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999a), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring

substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

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

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

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

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

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

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

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

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

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

Genotoxicity

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

Specifications

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

Structural Relationship

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

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

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		24

The present revision of FGE.78, Revision 1 concerns the re-consideration of one JECFA evaluated substance considered in FGE.78.

In FGE.78, which contains 24 aliphatic, alicyclic and aromatic hydrocarbons, 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 are required.

Additional data (long term study of toxicity, mutagenicity studies and new tonnage figure) have now become available for beta-myrcene [FL-no: 01.008] and the present revision of FGE.78, FGE.78Rev1 includes the evaluation of these data submitted by the Industry (Flavour Industry, 2010a).

After publication of FGE.78, the JECFA has re-evaluated flavouring substances for which estimated intake was originally based on anticipated poundage data (JECFA, 2009c), but for which new tonnage data were submitted to the JECFA by Industry. These new tonnage figures are included in the present FGE for [FL-no: 01.029 and 01.040].

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. 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 and another 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). This consideration will therefore deal with 24 JECFA evaluated aliphatic, alicyclic and aromatic hydrocarbons.

1.1.2. EFSA Considerations

The Panel concluded that all the 24 substances in the JECFA flavouring group of aliphatic, alicyclic and aromatic hydrocarbons are structurally related to the group of aliphatic and aromatic hydrocarbons evaluated by EFSA in the Flavouring Group Evaluation 25, Revision 2 (FGE.25Rev2).
Because of the structural diversity within this group, EFSA subdivided the substances considered in FGE.25Rev2 into eight 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. Some additional subgroups have been created for substances which do not bear structural similarity to any of the flavouring substances considered in FGE.25, Revision 2 (e.g. the biphenyls). The subgrouping has been shown in Table 4.1 in Section 4.3.

1.2. Isomers

1.2.1. JECFA 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 24 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.

1.2.2. EFSA Considerations

Information is lacking about the isomerism for the two substances [FL-no: 01.018, and 01.061] and about the composition of the mixture of 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].

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all 24 substances (JECFA, 2005b) (see Table 1).

1.3.2. EFSA Considerations

The available specifications are considered adequate for 10 substances. Information on isomerism and/or information on composition of mixture is lacking 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] (see Section 1.2).

2. Intake Estimations

2.1. JECFA Status

For 23 of the 24 substances evaluated through the JECFA Procedure intake data are available for the EU (see Table 3.1). For the remaining substance production figure is only available for the USA.

2.2. EFSA Considerations

For one substance [FL-no: 01.024] no production figure is available for Europe, so a MSDI value for the EU cannot be calculated for this substance.

Only for two of the 24 JECFA evaluated substances normal and maximum use levels have been provided by the Flavour Industry [FL-no: 01.008 and 01.026] (EFFA, 2005a) (see Table 2.2.1). Based on these normal use levels, mTAMDI figure (see Table 2.2.2) can be calculated (EC, 2000a; EFSA, 2004d).

Table 2.2.1 Normal and Maximum use levels (mg/kg) available for JECFA evaluated substances in FGE.78Rev1

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

Table 2.2.2 Estimated intakes based on the MSDI and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/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	ND	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
01.008	Myrcene	290	153	3900	Class I	1800
01.004	Pin-2(3)-ene	1800	2444		Class I	1800
01.045	d-Limonene	34000	12726		Class I	1800
01.011	4-Methyl-1,1'-biphenyl	0.0085	0.08		Class III	90
01.013	Biphenyl	0.00085	0.7		Class III	90
01.014	1-Methylnaphthalene	0.73	0.06		Class III	90

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[4] from the JECFA (JECFA, 2006a)

Aliphatic and alicyclic hydrocarbons

In vitro

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

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), beta-myrcene (FL-no: [FL-no: 01.008]; up to 10,000 µg/plate), α-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, 1983a; Haworth et al., 1983; Jagannath, 1984a; Jagannath, 1984b; Connor et al., 1985; Heck et al., 1989; NTP, 1990e; 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, 1990e). 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, 1990e). 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).

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 beta-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 alpha-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 urineether extract. The urine samples of rats treated with alpha-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, delta-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), biphenyl ([FL-no: 01.013]; up to 10,000 µg/plate), 4-methylbiphenyl ([FL-no: 01.011]; up to 1000 µg/plate), or 1-methylnaphthalene ([FL-no: 01.014]; up to 4266 µg/plate) were incubated with *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537, TA1538, and/or TA1532, TA2636, TA2637, G46, C3076, or D3052 with and without metabolic activation (Clark et al., 1977; Clark et al., 1979; Anderson and Styles, 1978; Rockwell and Raw, 1979; Florin et al., 1980; Hirayama et al., 1981; Probst et al., 1981; Haworth et al., 1983) and (Pagano et al., 1983; Nohmi et al., 1985; Brams et al., 1987; Houk et al., 1989; NTP, 2004i; NTP, 2004j; NTP, 2004k; Zeiger et al., 1992)). Biphenyl also gave negative results when incubated with *Escherichia coli* strains WP2 and WP2 uvrA- in the modified Ames test (Probst et al., 1981), strain PQ37 in the SOS chromotest (up to 154 µg/ml) (Brams et al., 1987), and with strains WP2, WP2 *uvrA*, WP100, and CM571 in a test for DNA repair (up to 4000 µg/disk) (Hirayama et al., 1981).

In contrast to the negative results obtained for biphenyl in *S. typhimurium* and *E. coli* systems, biphenyl [FL-no: 01.013] produced genetic effects in an assay with *Saccharomyces cerevisiae* strain D7, with and without metabolic activation, at concentrations of up to 1 mmol/l (Pagano et al., 1983). In an assay for forward mutation in mouse lymphoma cells, biphenyl produced significant increases in mutation frequency in L5178Y cells at concentrations of 45.6 - 60.9 µg/ml without metabolic activation, and 3.1 - 9.3 µg/ml with activation (Wangenheim and Bolcsfoldi, 1988). However, the increases were ≥ 2-fold only at 60.9 µg/ml without activation, and 6.2 - 9.3 µg/ml with activation. At these concentrations, cell viability was ≤ 15 %. At lower concentrations of 15.2 - 30.4 µg/ml without metabolic activation, and 0.8 - 1.5 µg/ml with activation, biphenyl gave negative results (Wangenheim and Bolcsfoldi, 1988). Cell viability was much higher at these lower concentrations (at least 49 %). Biphenyl did not induce sister chromatid exchanges (SCE) or chromosomal aberrations in Chinese hamster Don cells at concentrations of up to 154 µg/ ml without metabolic activation (Abe & Sasaki, 1977), nor did it induce unscheduled DNA synthesis in rat hepatocytes at concentrations of 0.002 - 154 µg/ml (Brouns et al., 1979; Probst et al., 1981; Hsia et al., 1983).

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 h. 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 beta-glucuronidase (Rockwell and Raw, 1979).

Conclusion on genotoxicity on aromatic hydrocarbons

Four substances in this group of flavouring agents have been tested in the Ames assay and found not to be mutagenic *in vitro* in bacteria. In addition to showing no mutagenic potential in the Ames assay,

biphenyl produced negative results in *E. coli* in the SOS chromotest and DNA repair test. On the other hand, biphenyl produced genetic effects in yeast (*S. cerevisiae*). In mammalian cell systems, negative results were obtained for biphenyl with respect to induction of SCE, chromosomal aberration, and unscheduled DNA synthesis. The positive finding for biphenyl in an assay for forward mutation in mouse lymphoma cells was obtained at near lethal concentrations. 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 JECFA, see Table 2.1 and 2.2.

3.2. Genotoxicity Studies - Text Taken[5] from EFSA FGE.25Rev2 (EFSA, 2011k)

In vitro / in vivo

Data from *in vitro* tests are available for six candidate substances, subgroup I: [FL-no: 01.038] and [FL-no: 01.057]; subgroup II: [FL-no: 01.037]; subgroup IVb: [FL-no: 01.051 and 01.053]; subgroup V[6]: [FL-no: 01.047] and 11 supporting substances, one from subgroup II, four from subgroup III, one from subgroup IVb, five from subgroup V (for one of these [FL-no: 01.004] also data for separate stereoisomers were available (+ and -)-alpha-pinene (pin-2(3)-ene)(isomer of [FL-no: 01.004]), and one structurally related substance (2-methylbuta-1,3-diene (isoprene)) from subgroup II. Data for two of the candidate substances [FL-no: 01.051 and 01.053] (subgroup IVb), data for four supporting substances [FL-no: 01.008] (subgroup II), [FL-no: 01.019] (subgroup III), [FL-no: 01.014] (subgroup IVb) [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 one candidate substance, *subgroup IVb*: [FL-no: 01.053], 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).

There are no data available on candidate or supporting substances from subgroups IVa, IVc and VI.

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 six candidate substances in subgroup II [FL-no: 01.032, 01.035, 01.037, 01.064, 01.070 and 01.078] there are, except for one negative bacterial reverse gene mutation test ("Ames test"), no genotoxicity data available.

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

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

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, 1999a).

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, 1999a). Isoprene has been classified in the EU as a *'Muta. Cat. 3; R68'* and *'Carc. Cat. 2; R45'* (ECB, 2005).

The available data on *in vivo* genotoxicity of 2-methylbuta-1,3-diene (synonym: isoprene) indicate a genotoxic potential of the substance. In the light of the evidence of carcinogenic activity of isoprene in rats and mice (NTP, 1999d) 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 beta-myrcene, several *in vitro* genotoxicity tests and three *in* vivo genotoxicity studies were available. All the *in vitro* genotoxicity tests on beta-myrcene were negative. Two micronucleus tests on peripheral blood cells and one chromosomal aberration assay with beta-myrcene gave negative results.

Conclusion on Genotoxicity for subgroup II

The structurally related substance myrcene [FL-no: 01.008] has like former Register substance 2-methyl-1,3-butadiene (isoprene), two conjugated terminal double bonds, but has, similar to candidate substance [Fl no: 01.064], a longer chain length, with 10 carbon atoms. In contrast to isoprene, the candidate substances in subgroup II do not contain conjugated terminal double bonds, except [FL-no: 01.064], which, however, are very structurally related to myrcene [FL-no: 01.008]. Therefore, the Panel considers myrcene a better supporting substance for the substances in group II than isoprene. 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 6 substances in subgroup II.

Subgroup III

For the six 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], alpha-terpinene [FL-no: 01.019] and alpha-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 IVb

Naphthalene

Naphthalene [FL-no: 01.053], which was negative in all bacterial gene mutation tests (Ames tests, Rec assay, Inductest) and in a unscheduled DNA synthesis (UDS) test in primary rat hepatocytes, gave a weakly positive result in a valid sister chromatid exchange (SCE) test both in the presence and absence of metabolic activation and a positive result in a valid chromosomal aberration test in the presence of S9. A positive result was also reported in a mammalian cell gene mutation test (mouse lymphoma assay). However, the validity of this study cannot be evaluated.

The available *in vivo* studies on the candidate substance naphthalene [FL-no: 01.053] reported negative results in a valid UDS test and in two micronucleus tests, for one of which the validity cannot be evaluated due to insufficiently reported experimental details.

The genotoxicity of naphthalene [FL-no: 01.053] has been evaluated by international expert bodies (WHO, 1998; EU-RAR, 2003; US ATSDR, 2005). Results of the *in vitro* genotoxicity studies that were evaluated are in line with the data summarised in the present evaluation. Negative results were reported for all the evaluated bacterial gene mutation tests (Ames tests, Rec assays, Inductests/SOS response tests). For *in vitro* mammalian gene mutation, cytogenetic, or DNA damage assays, equally mixed results were reported as in the present evaluation. In addition to the studies cited in the present evaluation, negative results were reported for mutations at the hprt and tk locus in a human B-lymphoblastoid cell line (Sasaki et al., 1997) and for single-strand breaks in an alkaline elution test with rat hepatocytes (Sina et al., 1983) as well as for some other less relevant endpoints (different cell transformation assays). Positive results were reported for naphthalene in a non-standard chromosomal aberration assay (Gollahon, 1991) and for the naphthalene metabolites 1,2- and 1,4-naphthoquinone in a SCE test (Wilson et al., 1996). For *in vivo* genotoxicity, besides the negative results from studies examining commonly accepted endpoints (micronuclei formation in mouse bone marrow, DNA single strand breaks and UDS in rat hepatocytes) as reported in the present evaluation, some positive results were reported for somatic mutations in *D. melanogaster* (Delgado-Rodriques et al., 1995), micronuclei in salamander larvae erythrocytes (Djomo et al., 1995), and DNA fragmentation in liver and brain tissue from mice and rats orally exposed to naphthalene (Bagchi et al., 2000). However, DNA fragmentation *per se* cannot be considered a specific endpoint of genotoxicity, being rather an indicator of cytotoxicity, in this case due to oxidative stress. Therefore, the study by Bagchi *et al*. (2000) has no relevance for the evaluation of the genotoxic potential of naphthalene. WHO (1998) noted that naphthalene was inactive in all short-term mutagenicity tests evaluated by IARC in 1983 (WHO, 1998). US ATSDR (2005) concluded that the available data suggest that genotoxic action by the naphthalene metabolite, 1,2-naphthoquinone, is plausible and that the mutagenic/genotoxic potential of naphthalene and its metabolites may be weak (US ATSDR, 2005). In the EU-RAR (2003) it was concluded that overall, the balance of evidence indicates that naphthalene is not genotoxic (EU-RAR, 2003).

2-Methylnaphthalene

The weak increases of chromosome aberrations (chromatid breaks only at the highest concentration) and of the SCE in cultured human lymphocytes in the presence of S9 are of doubtful relevance (Kulka et al., 1988). According to the authors, these effects do not indicate that 2-methylnaphthalene is a potentially genotoxic substance.

The genotoxicity of 2-methylnaphthalene has been evaluated by international expert bodies (EFSA, 2004e; US ATSDR, 2005). Results of the *in vitro* genotoxicity studies that were evaluated are in line with the data summarised in the present evaluation. Negative results were reported for all the evaluated bacterial gene mutation tests (Ames tests, Rec assays, Inductests/SOS response tests).

The supporting substance, 1-methylnaphthalene, gave a weak increase, of doubtful biological relevance, of SCE in cultured human lymphocytes in the presence of S9 (Kulka et al., 1988). This effect was interpreted by the authors not to be an indication of genotoxic potential. Furthermore, 1-methylnaphthalene gave negative results in two bacterial reverse gene mutation tests (Florin et al., 1980; Kaden et al., 1979); and in a chromosomal aberration assay in human lymphocytes (Kulka et al., 1988).

Conclusion on genotoxicity for subgroup IVb

For naphthalene there is indication of *in vitro* genotoxicity especially at chromosome level. However, this genotoxic activity is not expressed in valid *in vivo* assays covering different end-points (e.g. micronucleus, UDS and DNA single strand breaks).

The available data on 2-methylnaphthalene, limited to the *in vitro* data referred to in the present evaluation, were considered not to give evidence for a genotoxic activity.

The Panel concluded that the available genotoxicity data on naphthalene and 2-methylnaphthalene do not preclude an evaluation of these substances through the Procedure.

Subgroup V

One candidate substances longifolene [FL-no: 01.047] was tested *in vitro* for bacterial reverse gene mutations and gave negative results.

For all genotoxicity studies on supporting substances, only negative results were reported in the available studies except for delta-3-carene (see Table 2.3). Delta-3-carene was studied individually as a component in wood fumes and wood fume condensates (Kurttio et al., 1990). It was reported to be positive in TA100 and TA102 strains in an insufficiently reported bacterial reverse gene mutation test in the absence of metabolic activation at high concentrations only, while it was negative in the presence of metabolic activation.

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

Overall conclusion on genotoxicity:

Data on the genotoxicity of the flavouring substances in this group are limited and the genotoxicity could not be assessed adequately for these substances. For one structurally related substance, 2-methylbuta-1,3-diene (synonym: isoprene) there is evidence of an *in vivo* genotoxic potential. However, the Panel concluded that the available data do not preclude evaluating the 37 candidate substances using the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA see Table 2.3 and 2.4.

3.3. EFSA Considerations

The Panel agrees with the JECFA conclusions on genotoxicity, except that the Panel has concern with respect to *in vitro* genotoxicity (absence of valid *in vivo* genotoxicity studies) of biphenyl [FL-no: 01.013] and to 4-methyl-1,1-biphenyl [FL-no: 01.011] due to its structural relationship with biphenyl.

4. Application of the Procedure

4.1. Application of the Procedure to 24 Aliphatic, Alicyclic and Aromatic Hydrocarbons by the JECFA (JECFA, 2006a)

1.2.1. Aliphatic and alicyclic hydrocarbons

According to the JECFA all these 19 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 alpha-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 alpha-pinene [FL-no: 01.004] were available. *d*-Limonene shares structural characteristics with alpha-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 alpha-pinene the second substituent is a dimethyl-substituted methylene bridge. Based on these chemical structures, it would be predicted that the toxicity of alpha-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. Alpha-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 alpha-pinene to be used as a structural analogue for alpha-pinene at this step of the Procedure. The estimated current per capita intakes of alpha-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 alpha-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.

1.2.2. Aromatic hydrocarbons

According to the JECFA two of these substances [FL-no: 01.002 and 01.010] belong to structural class I and the remaining three [FL-no: 01.011, 01.013 and 01.014] to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded all five aromatic 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 classes I and III (step A3).

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

The evaluations of all 24 aliphatic, alicyclic and aromatic hydrocarbons are summarised in Table 3.1.

4.2. Application of the Procedure to Aliphatic and Aromatic Hydrocarbons in FGE.25Rev2

For the safety evaluation of the 37 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 37 substances are summarised in Table 2.

Step 1

Thirty-one of the 37 candidate substances evaluated using the Procedure are classified into structural class I [FL-no: 01.001, 01.022, 01.023, 01.027, 01.028, 01.030, 01.032, 01.033, 01.034, 01.035, 01.037, 01.038, 01.039, 01.042, 01.043, 01.044, 01.046, 01.047, 01.050, 01.052, 01.054, 01.055, 01.056, 01.057, 01.059, 01.060, 01.064, 01.066, 01.067, 01.070 and 01.078], two into structural class II [FL-no: 01.031 and 01.058], and four into structural class III, [FL-no: 01.021, 01.036, 01.051 and 01.053], 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 4.1) 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.

The remaining candidate substance from subgroup I [FL-no: 01.050] may be biotransformed to a neurotoxic gamma-diketone. Three candidate substances from subgroup II [FL-no: 01.037, 01.064 and 01.070] contain terminal double bonds in the absence of other functional groups that may provide alternative routes of detoxication. For the two candidate substances from subgroup IVb [FL-no: 01.051 and 01.053] it has been shown that they may be converted to toxic metabolites. Therefore, for these six 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.

For the remaining 21 candidate substances [FL-no: 01.021, 01.022, 01.023, 01.030, 01.031, 01.032, 01.035, 01.036, 01.042, 01.043, 01.044, 01.047, 01.052, 01.055, 01.056, 01.058, 01.059, 01.060, 01.066, 01.067 and 01.078] 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.

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 microgram (Table 3.2). These intakes are below the threshold of concern of 1800 microgram/person/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: 01.001and 01.046] have an estimated European daily *per capita* intake of 4000 and 2100, respectively, which are above the threshold of concern of 1800 microgram/person/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/day can be derived. The estimated daily *per capita* intake is 4000 microgram for [FL-no: 01.001] and 2100 microgram for [FL-no: 01.046], corresponding to 0.07 mg/kg bw/day and 0.035 mg/kg bw/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 21 candidate substances [FL-no: 01.022, 01.023, 01.030, 01.032, 01.035, 01.037, 01.042, 01.043, 01.044, 01.047, 01.050, 01.052, 01.055, 01.056, 01.059, 01.060, 01.064, 01.066, 01.067, 01.070 and 01.078] proceeding via the B-side and which have been assigned to structural class I have estimated European daily *per capita* intakes between 0.0085 and 28 microgram (Table 3.2). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I. Two of the candidate substances [FL-no: 01.031 and 01.058] proceeding via the B-side and assigned to structural class II, have estimated European daily *per capita* intakes of 0.0012 and 0.12 micrograms, respectively. These intakes are below the threshold of concern of 540 microgram/person/day for structural class II. Four candidate substances [FL-no: 01.021, 01.036, 01.051 and 01.053] proceeding via the B-side and assigned to structural class III, have European daily *per capita* intakes of 0.15, 1.2, 0.0012 and 0.013 microgram. These intakes are below the threshold of concern for structural class III of 90 microgram/person/day. Accordingly, these 27 substances all proceed to step B4 of the Procedure.

Step B4

No adequate No Observed Adverse Effect Levels (NOAELs) have been submitted for any of these 27 candidate substances at step B4 [FL-no: 01.021, 01.022, 01.023, 01.030, 01.031, 01.032, 01.035, 01.036, 01.037, 01.042, 01.043, 01.044, 01.047, 01.050, 01.051, 01.052, 01.053, 01.055, 01.056, 01.058, 01.059, 01.060, 01.064, 01.066, 01.067, 01.070 and 01.078] or for any structurally related substances. Therefore, the Panel concluded that additional data are required for these substances.

4.3. EFSA Considerations

New toxicity data on beta- myrcene was submitted by the Industry. These data have been evaluated in detail in FGE.25Rev2. In the evaluation of the new toxicity study of beta-myrcene it was concluded in FGE.25Rev2 that: "No overall NOAEL from the NTP study on beta-myrcene could be allocated due to the observation of renal toxicity in male and female rats at all dose groups. The Panel has considered deriving a BMDL from the NTP study of myrcene. However, a BMDL from this study could not be derived since nearly 100 % incidence of nephrosis was observed in male rats already at the lowest dose of beta-myrcene.

The 24 JECFA evaluated hydrocarbons and the 37 EFSA evaluated hydrocarbons are distributed into 10 subgroups of structurally related substances by EFSA. The Panel conclusions on predictions of metabolism are shown in Table 4.1 below.

Table 4.1 Subgroups as classified by EFSA within the groups of the JECFA evaluated hydrocarbons (JECFA, 2006a) and the EFSA evaluated hydrocarbons in FGE.25Rev2. The JECFA evaluated hydrocarbons are listed in brackets.

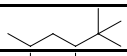
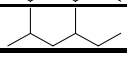
FL-no	EU Register name	Structural formula	Struc. class	Predicted metabolism to innocuous products? (by EFSA)	A- or B-side of the Procedure (EFSA)	A- or B-side of the Procedure (JECFA)
I: ACYCLIC ALKANES						
01.033	2,2-Dimethylhexane		I	Yes	A	
01.034	2,4-Dimethylhexane		I	Yes	A	

Table 4.1 Subgroups as classified by EFSA within the groups of the JECFA evaluated hydrocarbons (JECFA, 2006a) and the EFSA evaluated hydrocarbons in FGE.25Rev2. The JECFA evaluated hydrocarbons are listed in brackets.

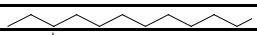
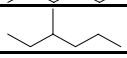
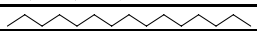
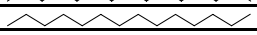
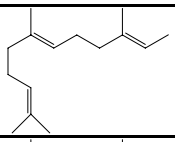
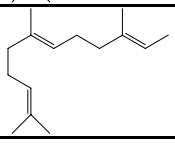
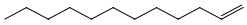
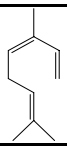
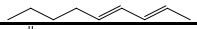
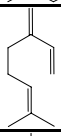
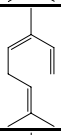
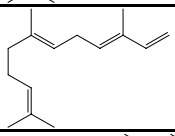
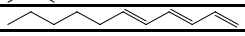
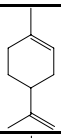
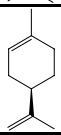
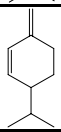
FL-no	EU Register name	Structural formula	Struc. class	Predicted metabolism to innocuous products? (by EFSA)	A- or B-side of the Procedure (EFSA)	A- or B-side of the Procedure (JECFA)
01.038	Dodecane		I	Yes	A	
01.050	3-Methylhexane		I	No (potential formation of neurotoxic gamma-diketone)	B	
01.054	Pentadecane		I	Yes	A	
01.057	Tetradecane		I	Yes	A	
II: ACYCLIC ALKENES						
01.032	2,3-Dihydrofarnesene		I	No (lack of supporting data)	B	
01.035	2,6-Dimethylocta-2,4,6-triene		I	No (lack of supporting data)	B	
01.037	Dodec-1-ene		I	No (presence of terminal double bond which may give rise to reactive metabolites without counteracting metabolic options)	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	
01.078	2,4-Nonadiene		I	No (lack of supporting data)	B	
(01.008)	(Myrcene)		I	No (lack of supporting data)	B	A
(01.018)	(beta-Ocimene)		I	No (lack of supporting data)	B	A
(01.040)	(alpha-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	
01.055	beta-Phellandrene		I	No (lack of supporting data)	B	

Table 4.1 Subgroups as classified by EFSA within the groups of the JECFA evaluated hydrocarbons (JECFA, 2006a) and the EFSA evaluated hydrocarbons in FGE.25Rev2. The JECFA evaluated hydrocarbons are listed in brackets.

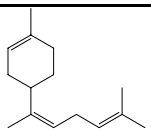
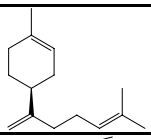
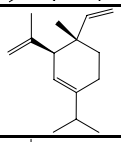
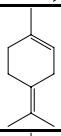
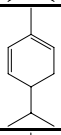
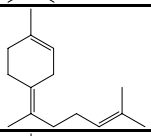
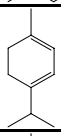
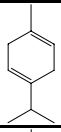
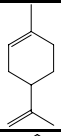
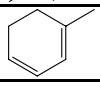
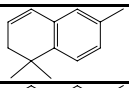
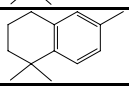
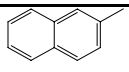
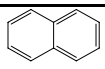
FL-no	EU Register name	Structural formula	Struc. class	Predicted metabolism to innocuous products? (by EFSA)	A- or B-side of the Procedure (EFSA)	A- or B-side of the Procedure (JECFA)
01.027	Bisabola-1,8,12-triene		I	Yes	A	
01.028	beta-Bisabolene		I	Yes	A	
01.039	delta-Elemene		I	Yes	A	
(01.005)	(Terpinolene)		I		A	A
(01.006)	(alpha-Phellandrene)		I		A	A
(01.016)	(1,4(8),12-Bisabolatriene)		I		A	A
(01.019)	(alpha-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						
IVa: CYCLOALKYL SUBSTITUTED BENZENE HYDROCARBONS						
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene		II	No (lack of supporting data)	B	
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene		II	No (lack of supporting dat)	B	
IVb: NAPHTHALENE HYDROCARBONS						
01.051	2-Methylnaphthalene		III	No (known metabolism to toxic metabolites)	B	
01.053	Naphthalene		III	No (known metabolism to toxic metabolites)	B	

Table 4.1 Subgroups as classified by EFSA within the groups of the JECFA evaluated hydrocarbons (JECFA, 2006a) and the EFSA evaluated hydrocarbons in FGE.25Rev2. The JECFA evaluated hydrocarbons are listed in brackets.

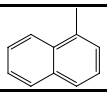
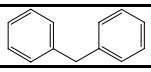
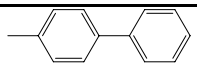
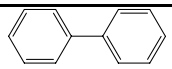
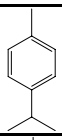
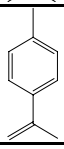
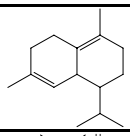
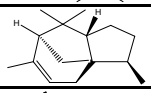
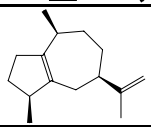
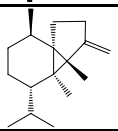
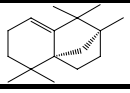
FL-no	EU Register name	Structural formula	Struc. class	Predicted metabolism to innocuous products? (by EFSA)	A- or B-side of the Procedure (EFSA)	A- or B-side of the Procedure (JECFA)
(01.014)	(1-Methylnaphthalene)		III	No (structurally related to FL-no: 01.051 and 01.053)	B	A
IVc: DIPHENYLMETHANE						
01.036	Diphenylmethane		III	No (lack of supporting data)	B	
IVd: BIPHENYLS						
(01.011)	(4-methyl-1,1-biphenyl)		III	No (anticipated to be metabolised to reactive metabolites responsible for toxicity)	Not evaluated through the Procedure. Genotoxic *in vitro* and unresolved problems with potential carcinogenicity	A
(01.013)	(Biphenyl)		III	No (anticipated to be metabolised to reactive metabolites responsible for toxicity)	Not evaluated through the Procedure. Genotoxic *in vitro* and unresolved problems with potential carcinogenicity	A
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						
01.021	Delta-Cadinene		III	No (lack of supporting data)	B	
01.022	alpha-Cedrene		I	No (lack of supporting data)	B	
01.023	1(5),11-Guaiadiene		I	No (lack of supporting data)	B	
01.030	beta-Cubebene		I	No (lack of supporting data)	B	
01.044	Isolongifolene		I	No (lack of supporting data)	B	

Table 4.1 Subgroups as classified by EFSA within the groups of the JECFA evaluated hydrocarbons (JECFA, 2006a) and the EFSA evaluated hydrocarbons in FGE.25Rev2. The JECFA evaluated hydrocarbons are listed in brackets.

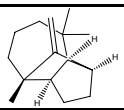
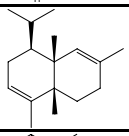
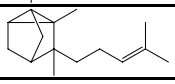
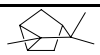
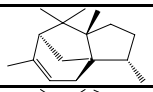
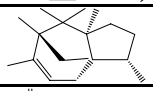
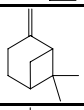
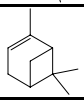
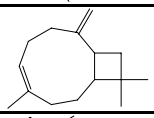
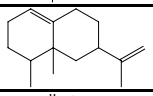
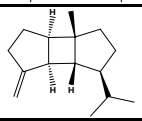
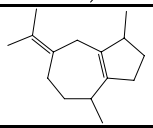
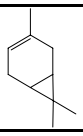
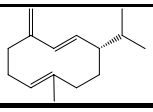
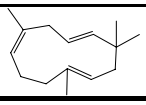
FL-no	EU Register name	Structural formula	Struc. class	Predicted metabolism to innocuous products? (by EFSA)	A- or B-side of the Procedure (EFSA)	A- or B-side of the Procedure (JECFA)
01.047	Longifolene		I	No (lack of supporting data)	B	
01.052	alpha-Muurolene		I	No (lack of supporting data)	B	
01.056	alpha-Santalene		I	No (lack of supporting data)	B	
01.059	4(10)-Thujene			No (lack of supporting data)	B	
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane		I	No (lack of supporting data)	B	
01.066	2-Cedrene		I	No (lack of supporting data)	B	
01.067	8(14)-Cedrene		I	No (lack of supporting data)	B	
(01.003)	(Pin-2(10)-ene)		I	No (lack of supporting data)	B	A
(01.004)	(Pin-2(3)-ene)		I	No (lack of supporting data)	B	A
(01.007)	(beta-Caryophyllene)		I	No (lack of supporting data)	B	A
(01.009)	(Camphene)		I	No (lack of supporting data)	B	A
(01.017)	(Valencene)		I	No (lack of supporting data)	B	A
(01.024)	(beta-Bourbonene)		I	No (lack of supporting data)	B	A
(01.026)	(1(5),7(11)-Guaiadiene)		I	No (lack of supporting data)	B	A
(01.029)	(delta-3-Carene)		I	No (lack of supporting data)	B	A
VI: MACROCYCLIC, NONAROMATIC HYDROCARBONS						
01.042	Germacra-1(10),4(14),5-triene		I	No (lack of supporting data)	B	

Table 4.1 Subgroups as classified by EFSA within the groups of the JECFA evaluated hydrocarbons (JECFA, 2006a) and the EFSA evaluated hydrocarbons in FGE.25Rev2. 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)
01.043	3,7,10-Humulatriene		I	No (lack of supporting data)	B	

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 16 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 the two biphenyls in subgroup IVd (biphenyl [FL-no: 01.013] and 4-methyl-1,1-biphenyl [FL-no: 01.011]), the JECFA considered a long-term carcinogenicity study in rats, which shows tumour related lesions in the bladder at doses of 4500 mg/kg bw/day in both sexes and tumours in males rats at this dose. The JECFA concluded that the induction of bladder tumours and tumour related lesions was secondary to the formation of bladder calculi. While this effect may be relevant for humans, it would be considered unlikely as the high doses necessary would not be applicable for human intakes and physiological differences between humans and rodents mean that humans are more likely to excrete bladder calculi and a NOEL of 25 mg/kg bw/day was derived from this study. However, the Panel concluded that the Procedure could not be applied for these two substances [FL-no: 01.011 and 01.013] based on *in vitro* genotoxicity and carcinogenicity data available. For biphenyl [FL-no: 01.013] the Panel concluded, due to concern with respect to *in vitro* genotoxicity (absence of valid *in vivo* genotoxicity) and unresolved problems with respect to carcinogenicity (especially development of bladder tumours in male rats), not to evaluate biphenyl [FL-no: 01.013] and the structurally related 4-methyl-1,1-biphenyl [FL-no: 01.011] through the Procedure. This is in accordance with the conclusions in the Concise International Chemical Assessment Document 6 (CICAD, 1999) concerning potential genotoxicity and carcinogenicity of biphenyl: "The results of *in vitro* studies indicate that biphenyl has mutagenic potential; in the absence of reassurance from reliable results from *in vivo* tests, it is assumed that exposure to biphenyl may be associated with a mutagenic risk" and "However, 1) observations of an increased incidence of histopathological effects and the formation of calculi within the urinary bladder, in the absence of bladder tumours, in female rats administered biphenyl for 2 years, 2) a lack of data identifying a direct association between calculi formation, regenerative hyperplasia of the urothelium, and the development of bladder tumours within individual male animals, and 3) the potential genotoxicity of biphenyl could suggest that the development of bladder tumours in the male rats may not have been entirely due to effects associated with the formation of calculi within the urinary bladder. This observation, as well as evidence of hepatocarcinogenicity in female mice, raises some concerns with respect to the potential carcinogenicity of biphenyl" (CICAD, 1999).

The Panel noted that the Joint Meeting of the FAO Working Party and the WHO Expert Committee on Pesticide Residues (FAO/WHO) has allocated an ADI of 0 - 0.125 mg/kg bw/day for biphenyl (FAO/WHO, 1967; JMPR, 1968). However, due to more recent studies on genotoxicity and carcinogenicity of biphenyl, the Panel has expressed concern with respect to the use of biphenyl as a flavouring substance (see above).

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. No adequate NOAEL was available, either for the JECFA or for the EFSA evaluated substances, to provide a margin of safety for the four substances at step B4, therefore additional data are required.

For one substance in subgroup IVb: Naphthalene hydrocarbons, metabolism to toxic metabolites are known [FL-no: 01.014]. No adequate NOAEL was available, either for the JECFA or the EFSA evaluated substances, to provide a margin of safety for the substance at step B4, therefore additional data are required.

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/day could be established. Compared to the MSDI of 18 microgram/*capita*/day ~ 0.3 µg/kg bw/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. No adequate NOAEL was available, either for the JECFA or the EFSA evaluated substances, to provide a margin of safety for the four substances at step B4, therefore additional data are required for these eight substances.

However, for one substance [FL-no: 01.024] no European production figure was available and consequently no European exposure estimate could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for this substance.

5. Conclusion

The Panel concluded that the 24 substances in the JECFA flavouring groups of aliphatic and alicyclic and aromatic hydrocarbons are structurally related to the group of 37 aliphatic and aromatic hydrocarbons evaluated by EFSA in the Flavouring Group Evaluation 25 (FGE.25).

The Panel agrees with the application of the Procedure as performed by the JECFA for eight of the 24 substances considered in this FGE [FL-no: 01.002, 01.005, 01.006, 01.016, 01.019, 01.020, 01.045 and 01.077].

Two substances [FL-no: 01.011 and 01.013] are genotoxic *in vitro* and there are unresolved problems with potential carcinogenicity and therefore the Panel concluded that they cannot be evaluated using the Procedure.

For the following 14 substances [FL-no: 01.003, 01.004, 01.007, 01.008, 01.009, 01.010, 01.014, 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 13 substances no applicable NOAEL is available for the substance itself or on a structurally related compound and therefore further data are required.

For one substances [FL-no: 01.024] the JECFA evaluation is only based on MSDI values derived from production figure from the USA. EU production figure is needed in order to finalise the evaluation of this substance.

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 structural class I. For these two substances 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 22 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 24 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 10 of the 24 JECFA evaluated substances. For two substances [FL-no: 01.018 and 01.061] information on the isomeric composition is lacking and 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] further information on the composition of the mixture is requested.

Thus, 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] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing information on stereoisomerism/composition of mixture). For two of the 24 JECFA evaluated substances the Procedure could not be applied [FL-no: 01.011 and 01.013] due to concern with respect to genotoxicity and carcinogenicity. For 13 of the 24 JECFA evaluated aliphatic, alicyclic and aromatic hydrocarbons [FL-no: 01.003, 01.004, 01.007, 01.008, 01.009, 01.014, 01.017, 01.018, 01.024, 01.026, 01.029, 01.040 and 01.061] additional toxicity data are requested. For the remaining six substances [FL-no: 01.002, 01.005, 01.006, 01.010, 01.016 and 01.077] the Panel agrees with JECFA conclusion “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY

Table 1: specifications summary for the JECFA evaluated substances in the present group (JECFA, 2005b)

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Aliphatic, Alicyclic and Aromatic Hydrocarbons (JECFA, 2005b)

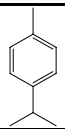
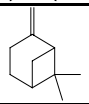
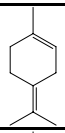
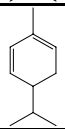
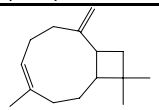
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
01.002 1325	1-Isopropyl-4-methylbenzene		2356 620 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, beta pinene and other common $C_{10}H_{16}$ terpenes". Composition of mixture to be specified.
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.
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	CASrn refers to (1R,4E,9S)-isomer. According to JECFA: Min. Assay value is "80 %" and secondary components "$C_{15}H_{24}$ terpene hydrocarbons (eg. Valencene)".

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Aliphatic, Alicyclic and Aromatic Hydrocarbons (JECFA, 2005b)

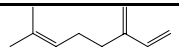
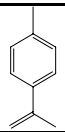
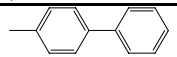
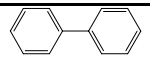
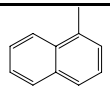
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
								Composition of mixture to be specified.
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	According to JECFA: Min. assay value is 90 % and secondary components "$C_{15}H_{24}$ terpene hydrocarbons (eg. Valencene); Min. assay may include traces of limonene, alpha and beta pinene and other common $C_{10}H_{16}$ terpenes". Composition of mixture to be specified.
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. The JECFA: Min. assay value is 80 % and sec. comp. "$C_{15}H_{24}$ terpene hydrocarbons (Valencene); Min assay may incl traces of limonene, myrcene, alpha- and beta-pinene and other common $C_{10}H_{16}$ terp". Composition of mixture to be specified.
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.	
01.013 1332	Biphenyl		3129 10978 92-52-4	Solid $C_{12}H_{10}$ 154.21	Insoluble Soluble	254 69 NMR 99 %	n.a. n.a.	
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	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Aliphatic, Alicyclic and Aromatic Hydrocarbons (JECFA, 2005b)

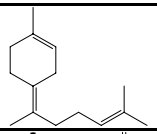
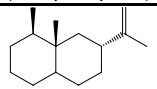
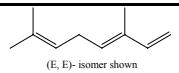
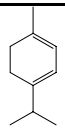
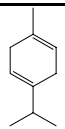
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
01.016 1336	1,4(8),12-Bisabolatriene		3331 10979 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. According to the JECFA: Min. assay value is "94 %" and secondary components "Other sesquiterpenes". Composition of mixture to be specified.
01.018 1338	beta-Ocimene 6)	 (E, 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	CASrn in Register does not specify stereoisomeric composition. Composition of mixture to be specified.
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	According to the JECFA: Min. assay value is "89 %" and secondary components "1,4- and 1,8-cineole; Minimum assay value may include traces of limonene, alpha- and beta-pinene and other common $C_{10}H_{16}$ terpenes". Composition of mixture to be specified.
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 C10H16 terpenes". Composition of mixture to be specified.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Aliphatic, Alicyclic and Aromatic Hydrocarbons (JECFA, 2005b)

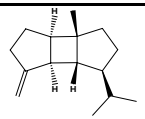
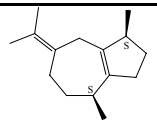
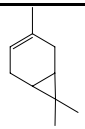
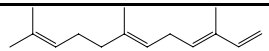
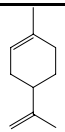
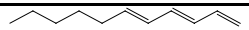
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
01.024 1345	beta-Bourbonene		 11931 5208-59-3	Liquid $C_{15}H_{24}$ 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)". Composition of mixture to be specified.
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)". Composition of mixture to be specified.
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. According to JECFA: Min. Assay value is "92 %" and secondary components "beta-pinene, limonene, myrcene, p-cymene". Composition of mixture to be specified.
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	CASrn & name in Register: (E,E). According to the JECFA: Min. assay value is "38 % alpha + 29 % beta (sum of E/Z isomers)" and sec.comp. "bisabolene, up to 10 % other isomers (valencene, bourbonene, cadinene, guaiene)". Composition of mixture to be specified.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Aliphatic, Alicyclic and Aromatic Hydrocarbons (JECFA, 2005b)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
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". Composition of mixture to be specified.
01.061 1341	Undeca-1,3,5-triene 6)		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	According to JECFA: Min. assay value is "94 % (sum of cis/trans isomers)" and secondary components "2,4,6-undecatriene (Z,Z,E)" CASrn in Register does not specify stereoisomers. Composition of mixture to be specified.
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	

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) Stereoisomeric composition not specified.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (in vitro / in vivo) for 19 Aliphatic, Alicyclic Hydrocarbons (JECFA, 2006a)

Table 2.1: Summary of Genotoxicity Data of 19 Aliphatic, Alicyclic Hydrocarbons

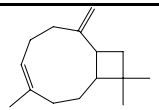
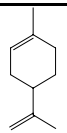
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
01.009 1323	Camphene		Reverse mutation	*S. typhimurium* TA98, TA100	0.05–100 µl/plate (42.1–84,200 µg/plate)[l]	Negative[b]	(Rockwell and Raw, 1979)
			Reverse mutation	*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	10–1000 µmol/l (1.4–136.2 µg/ml)[d,e]	Negative[f]	(Sasaki et al., 1989)
01.007 1324	beta-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, 1984b)
			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	10–1000 µmol/l (2.0–204.4 µg/ml)[i,e]	Negative[f]	(Sasaki et al., 1989)
			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, 1990e)
			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)

Table 2.1: Summary of Genotoxicity Data of 19 Aliphatic, Alicyclic Hydrocarbons

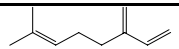
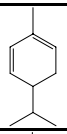
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	*S. typhimurium* TA102	Up to 5 000 µg/plate	Negative[b]	(Müller et al., 1993)
			Forward mutation, (non-)reciprocal recombination	*Saccharomyces cerevisiae MP1*	Up to 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, 1990e)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	10–333 µmol/l (1.4–45.4 µg/ml)[k,e]	Negative[f]	(Sasaki et al., 1989)
			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, 1990e)
			Chromosomal aberration	Chinese hamster ovary cells	10–100 µg/ml -S9; 50–500 µg/ml +S9	Negative[c]	(Anderson et al., 1990; NTP, 1990e)
			Cell transformation	Syrian hamster embryo cells	0.1–100 µg/ml	Negative	(Pienta, 1980)
			Cell transformation	Syrian hamster embryo cells	0.1–3 mmol/l (13.6–408.7 µg/ml)[k]	Positive[n]	(Rivedal et al., 2000)
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	alpha-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)
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,	0.1–25 µl/plate (85.9–21 480 µg/plate)[r,t]	Negative[c]	(Jagannath, 1984a)

Table 2.1: Summary of Genotoxicity Data of 19 Aliphatic, Alicyclic Hydrocarbons

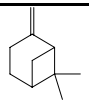
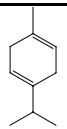
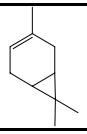
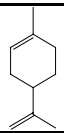
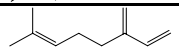
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
				TA1538			
			Reverse mutation	*S. typhimurium* TA98, TA100, UTH8413, UTH8414	10–500 µg/plate	Negative[c]	(Connor et al., 1985)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, 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, 1983a)
			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	delta-3-Carene		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	1.25–5 µl/plate (1 078–4 314 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)
			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)

Table 2.1: Summary of Genotoxicity Data of 19 Aliphatic, Alicyclic Hydrocarbons

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			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[e]	(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 α-pinene of 0.8592 g/ml (Lewis, 1999).

s Calculated using relative molecular mass of a-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 δ-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 2.2: Genotoxicity Data (in vitro / in vivo) for Five Aromatic Hydrocarbons (JECFA, 2006a)

Table 2.2: Summary of Genotoxicity Data of Five Aromatic Hydrocarbons

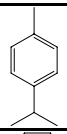
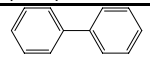
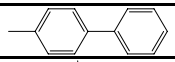
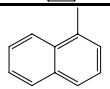
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
01.002 1325	1-Isopropyl-4-methylbenzene		Reverse mutation	*S. typhimurium* TA98, TA100	0.05–100 µl/plate (42.7–85 300 µg/ plate)[a]	Negative[b]	(Rockwell and Raw, 1979)
01.013 1332	Biphenyl		Reverse mutation	*S. typhimurium* TA98, TA1535, TA1537, TA1538	10–10 000 µg/plate[d]	Negative[e]	(Clark et al., 1977)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1538	4–2500 µg/plate	Negative[b]	(Anderson and Styles, 1978)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1–10 000 µg/plate	Negative[e]	(Clark et al., 1979)
			Reverse mutation	*S. typhimurium* TA98, TA100	1–100 µg/plate	Negative[e]	(Hirayama et al., 1981)
			Reverse mutationf	*S. typhimurium* G46, TA98, TA100, TA1535, TA1537, TA1538, C3076, D3052; *Escherichia coli* WP2 and WP2uvrA-	NR[g]	Negative[e]	(Probst et al., 1981)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–100 µg/plate[h]	Negative[e]	(Haworth et al., 1983; NTP, 2004i; NTP, 2004j)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1532, TA1535, TA1537, TA1538, TA2636	0.1–500 µg/plate[i]	Negative[e]	(Pagano et al., 1983)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA2637	10–5000 µg/plate[j]	Negative[e]	(Nohmi et al., 1985)
			Reverse mutation	*S. typhimurium* TA97, TA98, TA100	1–100 µg/plate	Negative[e]	(Brams et al., 1987)
			Reverse mutation	S. typhimurium TA98, TA100	≤2000 µg/plate	Negative[e]	(Houk et al., 1989)
			SOS induction	*Escherichia coli*	2.4–154 µg/ml	Negative[e]	(Brams et al., 1987)
			DNA repair	*E. coli* WP2, WP2uvrA, CM571, WP100	4000 µg/disc	Negative	(Hirayama et al., 1981)
			Mitotic recombination, gene conversion,	*Saccharomyces cerevisiae*	≤1 mmol/l plate)[a] (154 µg/ml)[k]	Positive[e]	(Pagano et al., 1983)

Table 2.2: Summary of Genotoxicity Data of Five Aromatic Hydrocarbons

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			reversion	*D7*			
			Forward mutation	Mouse lymphoma L5178Y *Tk+/- cells*	98.7–395 µmol/l -S9 (15.2–60.9 µg/ml)[k,l]; 5.01–60 µmol/l +S9 (0.8–9.3 µg/ml)[k,m]	Positive[n]; Positive[o]	(Wangenheim and Bolcsfoldi, 1988)
			Sister chromatid exchange	Chinese hamster Don cells	0.1–1 mmol/l (15.4–154 µg/ml)[k]	Negative[p,q]	(Abe & Sasaki, 1977)
			Chromosomal aberration	Chinese hamster Don cells	0.1–1 mmol/l (15.4–154 µg/ml)[k]	Negative[p]	(Abe & Sasaki, 1977)
			Unscheduled DNA synthesis	Rat hepatocytes	0.01–1000 µmol/l (0.002–154 µg/ml)[k]	Negative	(Brouns et al., 1979)
			Unscheduled DNA synthesis	Rat hepatocytes	0.5–1000 nmol/ml (0.08–154 µg/ml)[k,r]	Negative	(Probst et al., 1981)
			Unscheduled DNA synthesis	Rat hepatocytes	0.1–100 µmol/l (0.02–15.4 µg/ml)[k]	Negative	(Hsia et al., 1983)
01.011 1334	4-Methyl-1,1'-biphenyl		Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535	0.1–10 µg/plate -S9[h]; 10–1000 µg/plate +S9[h]	Negative[e]	(Zeiger et al., 1992)
01.014 1335	1-Methylnaphthalene		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	0.03–30 µmol/plate (4.3–4266 µg/plate)[t,u]	Negative[e]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535	0.3–33 µg/plate -S9[h]; 1–100 µg/plate +S9	Negative[e]	(NTP, 2004k)

a Calculated using a density of p-cymene of 0.853 g/ml (Lewis, 1999).

b With metabolic activation.

c Dowtherm A, a mixture of diphenyl (26 %) and diphenyl oxide (72 %), was tested.

d Cytotoxicity observed at the highest dose/concentration tested.

e With and without metabolic activation.

f Modified Ames test.

g NR, not reported.

h Slight toxicity was occasionally observed at the highest concentration tested.

i Cytotoxicity observed at doses of 50 to 100 µg/plate, depending on the different tester strains.

j Lethality observed at doses of 2000 to 5000 µg/plate.

k Calculated using the relative molecular mass of biphenyl of 154.21.

l Cytotoxicity observed at 395 µmol/l (6 % cell viability), while 345 µmol/l is near-leathal concentration (14 % cell viability).

m 40 and 60 µmol/l are near-lethal concentrations (12 - 15 % cell viability).

n A significant increase in mutation frequency was noted at 296 - 395 µmol/l (with a twofold increase only at 395 µmol/l), but not at 98.7 - 197 µmol/l.

o Significant increase in mutation frequency was noted at 20–60 µmol/l (with an increase of more than twofold only at 40 and 60 µmol/l), but not at 5.01–10 µmol/l.

p Without metabolic activation.

[q] Significant increase in induction of sister chromatid exchange was noted, but the increase was not dose-dependent and was less than twice the control value. At the highest concentration (1 mmol/l), the mitotic index was decreased to > 50 % of that for controls.

[r] Cytotoxicity observed at concentrations > 100 nmol/ml.

[s] Mixed isomers of methylbiphenyl tested.

[t] Calculated using the relative molecular mass of 1-methylnaphthalene of 142.20.

[u] Cytotoxicity observed at doses >3 µmol/plate.

Table 2.3: Genotoxicity Data (in vitro) EFSA / FGE.25Rev2

In vitro mutagenicity/genotoxicity data are available for six candidate substances of the present flavouring group evaluation, for 11 supporting substances evaluated by the JECFA at the 63rd meeting and for two separate stereoisomers and for one structurally related non-Register substance *(2-Methylbuta-1,3-diene)*. Substances listed in brackets are the JECFA evaluated supporting substances in FGE.25Rev2

Table 2.3: GENOTOXICITY (*in vitro*)

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, 1999b)	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, 1999b)	Validity cannot be evaluated as substance is not specified. Cedarwood oil terpenes and tertonoids.
Longifolene [1.047]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537, TA102	1-5000	Negative[1]	(Sokolowski, 2001)	
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

Table 2.3: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						se, but increased the mutagenesis induced by pretreatment with the carcinogen.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; UTH8414; UTH8413	0, 50, 100, 500, 1000, 2000 µg/plate	Negative[1]	(Conner 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.
Dodec-1-ene [01.037]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 *E. coli* WP2uvrA	0.2 to 2000 µg/plate	Negative[1]	(Dean, 1980)	Unpublished GLP-study. IUCLID abstract available only. Details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
(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, 1999d)	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, 1999d; Galloway et al., 1987a)	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, 1999d; Galloway et al., 1987a)	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	100 - 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	100 - 1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange test	Human lymphocytes	100 - 1000 µg/ml	Negative[1]	(Kauderer et al., 1991)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells and hepatic tumour cell line	100 - 500 µg/ml	Negative[1]	(Röscheisen et al., 1991)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).

Table 2.3: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	*S. typhimurium* TA97a ; TA98; TA100; TA1535	Up to 1500 µg/plate (16 concentrations)	Negative	(Gomes-Carneiro et al., 2005a)	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, 2010b)	
	Reverse mutation	*E. coli* WP2*uvrA*/pKM101	50 – 10 000 µg/plate	Negative[1]	(NTP, 2010b)	
	Ames	*S. typhimurium* TA97a; TA98; TA100; TA1535	10 - 5000	Negative[1]	(Gomes-Carneiro et al., 2005a)	
	Ames	*S. typhimurium* TA97a; TA98; TA100; TA1535	1 - 1500	Negative[1]	(Gomes-Carneiro et al., 2005a)	
(*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	150,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test	*S. typhimurium* TA102	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	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, UTH8414 and UTH8413	0, 10 to 500 µg/plate (5 concentrations)	Negative[1]	(Conner 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 nl/ml	Negative[1]	(Myhr et al., 1990)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Chromosomal aberration assay	Chinese hamster ovary cells	Up to 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	Up to 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	10 - 333 µmol/ml (1.4 - 45.4 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	Uo to 50,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Unscheduled DNA synthesis	Rat hepatocytes	Uo to 30 µg/ml	Negative	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(alpha-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., 2005a)	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.
(alpha–Phellandrene [01.006])	Sister chromatid exchange test	Chinese hamster ovary cells	Uo to 1000 µM(136.2 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(delta-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.

Table 2.3: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Pin-2(3)-ene [01.004])	Ames test	*S. typhimurium* TA98; TA100	Up to 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	Up to 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	Up to 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; UTH8413; UTH8414	0, 10 to 500 µg/plate (5 concentrations)	Negative[1]	(Conner et al., 1985)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Unscheduled DNA synthesis	Rat hepatocytes	Up to 10000 µg/ml	Negative	(Heck et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(+)-alpha-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., 2005a)	Valid studies.
(-)-alpha-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., 2005a)	Valid studies.
(Pin-2(10)-ene [01.003])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 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).
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate (4290 µg/plate)	Negative[1]	(DeGraff, 1983a)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 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	0.05 - 100 µl/plate (42.1 - 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]	(Conner et al., 1985)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Sister chromatid exchange test	Chinese hamster ovary cells	10 - 1000 µM (1.4 - 136.2 µg/ml)	Negative	(Sasaki et al., 1989)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
(beta–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).
	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).
Naphthalene [01.053]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (3.9, 39, 385, 3850 g/plate)	Negative[1]	(Florin et al., 1980)	Published non-GLP study. Part of a larger mutagenicity screening study evaluating 239 compounds. Due to the limited report of experimental details and results the validity of the study cannot be evaluated. Cytotoxicity observed at doses >3 µM/plate.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	0, 3, 10, 30, 100, 300 µg/plate	Negative[1]	(Godek et al., 1985)	Unpublished GLP study carried out according to OECD guideline 471.

Table 2.3: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	0, 3, 10, 30, 100, 300 µg/plate	Negative[1]	(Stankowski, 1987)	Unpublished GLP study carried out according to OECD guideline 471. Repeat confirmation of Ames test by Godek *et al*, 1985.
	Ames test (preincubation method)	*S. typhimurium* TM677	0, 1, 2 mM (0, 128, 256 µg/ml)	Negative[2]	(Kaden et al., 1979)	Published non-GLP study of limited validity (only one strain, concentrations used were cytotoxic, insufficient report of experimental details and results).
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 100 µg/plate	Negative[2]	(McCann et al., 1975)	Published summary report of a large study evaluating the mutagenic potential of 300 chemicals. Due to the limited report of experimental details and results the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, (0.3), 1, 3.3, 10, 33, 100 µg/plate	Negative[1]	(Mortelmans et al., 1986) (NTP, 1992g)	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. Study design and detailed results on naphthalene also included in NTP, 1992g. In the absence of metabolic activation the concentration of 100 microgram/plate was completely toxic and not tested any more in the second trial when 0.3 microgram/plate was used as additional concentration. In the presence of metabolic activation the highest concentration was slightly toxic.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	0, 5, 10, 25, 50, 100, 300 µg/plate (-S9) 0, 10, 25, 50, 100, 300, 900 µg/plate (+ S9)	Negative[1]	(Lawlor, 1994)	Unpublished GLP study carried out in accordance with OECD guideline 471. Cytotoxicity was observed in a preliminary study at 66.7 microgram/plate and above in the absence of S9 mix and at 333 microgram/plate and above in the presence of S9 mix.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; UTH8414; UTH8413	0, 100, 500, 1000, 2000 µg/plate	Negative[1]	(Conner 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.
	Ames test (plate incorporation method)	*S. typhimurium* TA97; TA98; TA100	0, 5, 10, 50, 250 µg/plate	Negative[1]	(Sakai et al., 1985)	Published non-GLP study of acceptable quality. Cytotoxicity was observed at the highest concentration with complete toxicity in TA97 (+/-S9) and in TA100 (-S9) and a reduced number of mutants in TA100 (+S9) and in TA98 (+/-S9).
	Ames test (plate incorporation)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	0, 3.3 to 10,000 µg/plate	Negative[1]	(Longfellow, 1991)	Only summary from CCRIS database available from. Validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537 *E. coli* WP2*uvrA*	1 to 100 µg/plate 10 to 10,000 µg/plate	Negative[1]	(Japan Chemical Industry)	Only summary from CCRIS database available from. Validity of the study cannot be evaluated.
	Rec assay	*E. coli* WP2 and WP100 *uvrA*$^-$ *recA*$^-$	0 to 2000 µg/plate (≥ 4 concentrations)	Negative[2]	(Mamber et al., 1984)	Published non-GLP study with adequate study design, however, deficient in the report of some

Table 2.3: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						details on method and results (no single doses, no detailed results, no data on cytotoxicity reported).
	Inductest (prophage induction test)	*E. coli* GY5027 *envA*$^-$ *uvrB*$^-$ (λ); GY4015 amp^R	0 to 2000 µg/plate (≥ 4 concentrations)	Negative[2]	(Mamber et al., 1984)	Published non-GLP study with adequate study design, however, deficient in the report of some details on method and results (no single doses, no detailed results, no data on cytotoxicity reported).
	Unscheduled DNA synthesis test	Primary rat hepatocytes	0, 0.16, 0.5, 1.6, 5, 16, 50, 166, 500, 1666, 5000 µg/ml	Negative	(Barfknecht et al., 1985)	Unpublished GLP-study according to OECD guideline 482. Excessive cytotoxicity observed at 50 to 5000 microgram/ml.
	Sister chromatid exchange test	Chinese hamster ovary cells	0, 2.7, 9, 27, 45, 90 µg/ml (-S9) 0, 9. 15, 27, 90 µg/ml (+S9)	Positive[1]	(NTP, 1992g)	Valid study in accordance with OECD guideline 479. No data on cytotoxicity reported. According to the study protocol the highest dose chosen was limited by cytotoxicity. Significant dose-related increase in frequency of SCE at concentrations from 27 - 90 µg/ml (without metabolic activation) and 15 - 27 µg/ml (with metabolic activation). Maximum values for the percent increase in SCEs/chromosome in cultures exposed to naphthalene relative to those exposed to solvent of 40 and 50 % were reached at the highest dose tested in the presence and absence of S9, respectively, whereas values of 360 – 640 % were reached with the positive control mitomycin C. Result is considered positive by NTP since the increase over solvent control observed is ≥ 20 % (NTP, 1992g; Galloway *et al*., 1987). Results would be considered negative by UK HSE as the increase in SCEs per cell does not reach the required minimum of at least 100 %)(EU RAR, 2003).
	Sister chromatid exchange test	Human peripheral mononuclear leukocytes	100 µM (13 µg/ml)	Negative[1]	(Tingle et al., 1993; Wilson et al., 1995)	Published non-GLP study of limited validity (only one concentration tested). Naphthalene was not cytotoxic to the dividing lymphocytes with and without metabolic activation at the concentration tested.
	Mammalian cell gene mutation test (Mouse lymphoma assay)	Mouse lymphocytes L5178Y *tk*$^+$/*tk*$^-$	0, 22 to 87 µg/ml (-S9) 0, 8 to 30 µg/ml (+S9)	Negative[3] Positive[2]	(Longfellow, 1991)	Only summary from CCRIS database available. Validity of the study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster ovary cells	15 to 75 µg/ml (-S9) 30 to 67.5 µg/ml (+S9)	Negative[3] Positive[2]	(NTP, 1992g)	Study carried out in accordance with OECD guideline 473, except that data on cytotoxicity are not reported. According to the study protocol the highest dose chosen was limited by cytotoxicity. Study is considered valid. The structural aberrations did not include gaps. In the presence of S9 the percent of cells with structural aberrations was significantly ($p \leq 0.05$) elevated at all concentrations tested

Table 2.3: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						compared to controls and the increase was significantly dose-related ($p \leq 0.001$). A maximum of 32 % of cells with aberrations was reached at the highest concentration vs. 0 - 1.5 % in negative and up to 52 % in positive controls. Result is considered positive by NTP since a statistically significant difference is observed for two or more doses (Galloway et al., 1987a).
(1-Methylnaphthalene [01.014])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.3, 43, 427, 4266 µ g/plate)	Negative[1]	(Florin et al., 1980)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Ames test (preincubation method)	*S. typhimurium* TM677	0, 0.7, 3.5, 7 mM (0, 498, 995 µg/ml)	Negative[2]	(Kaden et al., 1979)	Abstracted by JECFA at their 63rd meeting (JECFA, 2006a).
	Chromosomal aberration assay	Human lymphocytes	0, 1, 2 mM (0, 142, 284 µg/ml) (-S9) 0, 0.25, 0.5, 1, 2 mM (0, 36, 71, 142, 284 µg/ml) (+S9)	Negative[1]	(Kulka et al., 1988)	Published non-GLP study largely in accordance with OECD guideline 473. Even if cytotoxicity data are not reported, the study is considered acceptable. The highest dose did not impair cell proliferation. No evidence for differences in the incidences of structural chromosomal aberrations (chromatid breaks, no exchanges seen) and gaps between treated and untreated cells ($p \leq 0.05$). 0.5 to 2.0 % of treated cells showed aberrations (gaps excluded) vs. 1 % of control cells.
	Sister chromatid exchange test	Human lymphocytes	0, 1, 2 mM (0, 142, 284 µg/ml) (-S9) 0, 0.25, 0.5, 1, 2 mM (0, 36, 71, 142, 284 µg/ml) (+S9)	Negative[3] Positive[2] (limited evidence)	(Kulka et al., 1988)	Published non-GLP study largely in accordance with OECD guideline 479. Cytotoxicity data not reported. The highest dose did not impair cell proliferation. In the presence of S9 the SCE frequency was significantly increased at each dose. An increase of 43 % was reported at the highest dose compared to the control. The effect was dose-related, but with a less marked increase at higher doses (saturation). According to OECD and NTP criteria the result in the presence of S9 is considered positive (significant increase, dose-relation, increase ≥ 20 % over solvent control). The authors of the study refer to the UK HSE guidelines on mutagenicity testing that require at least a doubling in SCE frequency for a positive response.
2-Methylnaphthalene [01.051]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0.03, 0.3, 3, 30 µM/plate (4.3, 43, 427, 4266 µg/plate)	Negative[1]	(Florin et al., 1980)	Published non-GLP study. Part of a larger mutagenicity screening study evaluating 239 compounds. Due to the limited report of experimental details and results the validity of the study cannot be evaluated. Bacterial toxicity observed at doses >3 µM/plate.
	Chromosomal aberration assay	Human lymphocytes	0, 2.0, 4.0 mM (0, 284,	Negative[3]	(Kulka et al., 1988)	Published non-GLP study largely in accordance

Table 2.3: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			569 microgram/ml) (-S9) 0, 0.25, 0.5, 1.0, 2.0, 4.0 mM (0, 35.6, 71.1, 142, 284 and 569 microgram/ml) (+S9)	 Positive[2] (limited evidence)		with OECD guideline 473. Even if cytotoxicity data are not reported, the study is considered acceptable. The highest dose did not impair cell proliferation. In the presence of S9 a statistically significant but weak increase (6.5fold above control) of structural aberrations (chromatid breaks, no exchanges seen) was observed at the highest tolerated dose (4 mM)The percent of cells with structural aberrations (excluding gaps) showed a dose-related increase (up to 5 % vs. 1.0 % in negative and 40 % in positive controls), however, the increase was not reported as statistically significant. A dose-dependent weak increase of gaps was also noted over the concentration range tested in the presence of S9. According to OECD and NTP criteria there is a weak evidence for a positive response since a statistically significant difference for structural aberrations per cell is observed for one dose (NTP, 1992g; (Galloway et al., 1987a) and the effect was dose-related (OECD guideline 473).
	Sister chromatid exchange test	Human lymphocytes	0, 2.0, 4.0 mM (0, 284, 569 microgram/ml) (-S9) 0, 0.25, 0.5, 1.0, 2.0, 4.0 mM (0, 36, 71, 142, 284 and 569 microgram/ml) (+S9)	Negative[3] Positive[2] (limited evidence)	(Kulka et al., 1988)	Published non-GLP study largely in accordance with OECD guideline 479. Even if cytotoxicity data are not reported, the study is considered acceptable. The highest dose did not impair cell proliferation. In the presence of S9 the SCE frequency was significantly increased at each dose. An increase of 80 % was reported at the highest dose compared to the control. The effect was dose-related. According to OECD and NTP criteria the result in the presence of S9 is considered positive (significant increase, dose-relation, increase ≥ 20 % over solvent control). The authors of the study refer to the UK HSE guidelines on mutagenicity testing that require at least a doubling in SCE frequency for a positive response.

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 2.4: Summary of Genotoxicity Data (in vivo) EFSA / FGE.25Rev2

In vivo mutagenicity/genotoxicity data are available for one candidate substance of the present flavouring group evaluation, for two supporting substances evaluated by JECFA at the 63rd meeting and for one structurally related *non-Register substance* (2-Methylbuta-1,3-diene). Substances listed in brackets are JECFA-evaluated substances.

Table 2.4: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(2-Methylbuta-1,3-diene)	*In vivo* Chromosomal aberration assay	Mouse (B6C3F1) bone marrow (male mice)	Inhalation	0, 438, 1750, 7000 ppm for 6 hours/day for 12 exposures over a period of 16 days (Trial 1) 0, 70, 220, 700 ppm for 6 hours/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 ppm for 6 hours/day for 12 exposures over a period of 16 days (Trial 1) 0, 70, 220, 700 ppm for 6 hours/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 ppm for 6 hours/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 ppm 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).
	In vivo Micronucleus test	Mouse (B6C3F1) peripheral blood cells	Gavage	0, 250, 500, 1000, 2000 mg/kg bw (single exposure)	Negative	(NTP, 2003c)	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, 2010b)	
(*d*-Limonene [01.045])	*In vivo* Comet assay	Mouse (ddY) / Rat (Wistar).	Oral	0, 2000 mg/kg	Negative	(Sekihashi et al., 2002)	
	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).

Table 2.4: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
	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/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.

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3.1: Summary of Safety Evaluation of 24 Aliphatic, Alicyclic and Aromatic Hydrocarbons (JECFA, 2005c)

Table 3.1: Summary of Safety Evaluation of Aliphatic, Alicyclic and Aromatic Hydrocarbons *(JECFA, 2005c))*

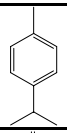
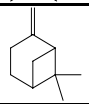
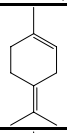
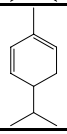
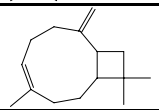
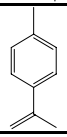
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)] (JECFA)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
01.002 1325	1-Isopropyl-4-methylbenzene		926 472	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
01.003 1330	Pin-2(10)-ene		1300 759	Class I A3: Intake below threshold	4)	Additional data required.	
01.005 1331	Terpinolene		660 70	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
01.006 1328	alpha-Phellandrene		79 410	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
01.007 1324	beta-Caryophyllene		330 508	Class I A3: Intake below threshold	4)	Additional data required.	Composition of mixture to be specified.
01.009 1323	Camphene		13 28	Class I A3: Intake below threshold	4)	Additional data required.	Composition of mixture to be specified.
01.010 1333	1-Isopropenyl-4-methylbenzene		18 0.3	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of Aliphatic, Alicyclic and Aromatic Hydrocarbons *(JECFA, 2005c))*

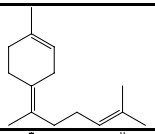
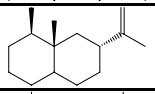
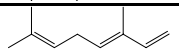
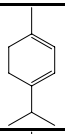
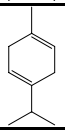
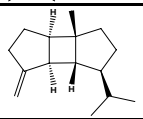
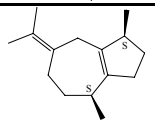
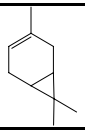
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)] (JECFA)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
01.016 1336	1,4(8),12-Bisabolatriene		13 10	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
01.017 1337	Valencene		53 26	Class I A3: Intake below threshold	4)	Additional data required.	Composition of mixture to be specified.
01.018 1338	beta-Ocimene	 (E, E)- isomer shown	55 11	Class I A3: Intake below threshold	4)	Additional data required.	Stereoisomeric composition to be specified. Composition of mixture to be specified.
01.019 1339	alpha-Terpinene		28 93	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	Composition of mixture to be specified.
01.020 1340	gamma-Terpinene		1200 321	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	Composition of mixture to be specified.
01.024 1345	beta-Bourbonene		ND 0.2	Class I A3: Intake below threshold	4)	Additional data required. MSDI based on USA production figure.	Composition of mixture to be specified MSDI based on USA production figure.
01.026 1347	1(5),7(11)-Guaiadiene		0.012 3	Class I A3: Intake below threshold	4)	Additional data required.	Composition of mixture to be specified.
01.029 1342	delta-3-Carene		290 40	Class I A3: Intake below threshold	4)	Additional data required.	Composition of mixture to be specified.

Table 3.1: Summary of Safety Evaluation of Aliphatic, Alicyclic and Aromatic Hydrocarbons *(JECFA, 2005c))*

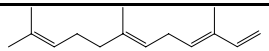
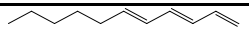
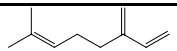
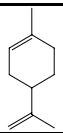
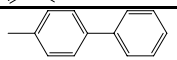
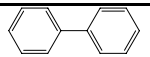
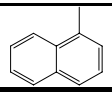
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)] (JECFA)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
01.040 1343	alpha-Farnesene		0.61 40	Class I A3: Intake below threshold	4)	Additional data required.	Composition of mixture to be specified.
01.061 1341	Undeca-1,3,5-triene		0.24 0.2	Class I A3: Intake below threshold	4)	Additional data required.	Stereoisomeric composition to be specified. Composition of mixture to be specified.
01.077 1344	1-Methyl-1,3-cyclohexadiene		0.012 313	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.
01.008 1327	Myrcene		290 153	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	Additional data required. The JECFA used a MSDI of 7100 µg/*capita*/day (New data received).	Composition of mixture to be specified.
01.004 1329	Pin-2(3)-ene		1800 2444	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	Additional data required.	Composition of mixture to be specified.
01.045 1326	d-Limonene		34000 12726	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	Composition of mixture to be specified.
01.011 1334	4-Methyl-1,1'-biphenyl		0.0085 0.08	Class III A3: Intake below threshold	4)	The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity/carcinogenicity.	
01.013 1332	Biphenyl		0.00085 0.7	Class III A3: Intake below threshold	4)	The Panel concluded that the substance cannot be evaluated through the Procedure due to concern	

Table 3.1: Summary of Safety Evaluation of Aliphatic, Alicyclic and Aromatic Hydrocarbons *(JECFA, 2005c))*

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)] (JECFA)	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
						with respect to genotoxicity/carcinogenicity.	
01.014 1335	1-Methylnaphthalene		0.73 0.06	Class III A3: Intake below threshold	4)	Additional data required.	

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

ND: not determined.

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.25Rev2)

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

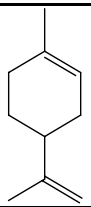
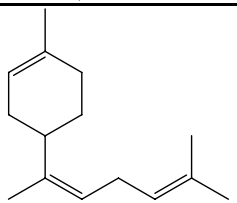
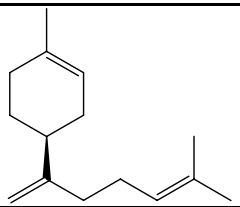
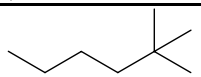
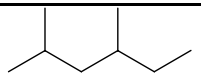
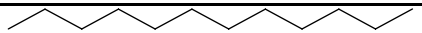
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
01.001	Limonene		4000	Class I A3: Intake above threshold; A5: Adequate NOAEL exist	4)	6)	
01.027	Bisabola-1,8,12-triene		0.024	Class I A3: Intake below threshold	4)	7)	
01.028	beta-Bisabolene		2.7	Class I A3: Intake below threshold	4)	6)	
01.033	2,2-Dimethylhexane		1.2	Class I A3: Intake below threshold	4)	6)	
01.034	2,4-Dimethylhexane		1.2	Class I A3: Intake below threshold	4)	6)	
01.038	Dodecane		0.012	Class I A3: Intake below threshold	4)	6)	

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

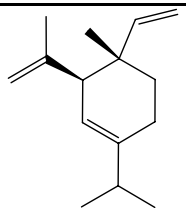
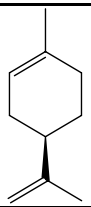
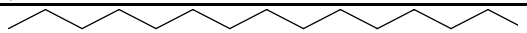
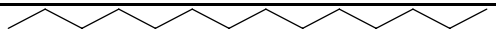
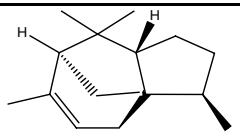
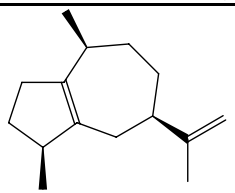
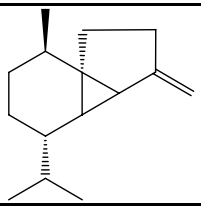
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
01.039	delta-Elemene		0.012	Class I A3: Intake below threshold	4)	6)	
01.046	l-Limonene		2100	Class I A3: Intake above threshold; A5: Adequate NOAEL exist	4)	6)	
01.054	Pentadecane		0.61	Class I A3: Intake below threshold	4)	6)	
01.057	Tetradecane		0.012	Class I A3: Intake below threshold	4)	6)	
01.022	alpha-Cedrene		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.023	1(5),11-Guaiadiene		1.2	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.030	beta-Cubebene		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

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

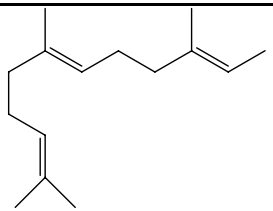
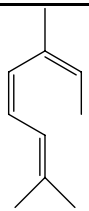
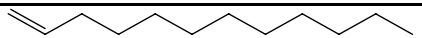
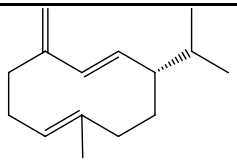
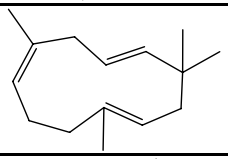
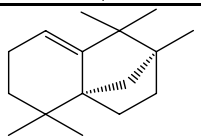
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
01.032	2,3-Dihydrofarnesene		0.12	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.035	2,6-Dimethylocta-2,4,6-triene		9.1	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.037	Dodec-1-ene		0.024	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.042	Germacra-1(10),4(14),5-triene		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.043	3,7,10-Humulatriene		1.2	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.044	Isolongifolene		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

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

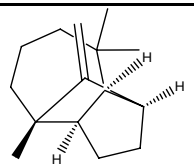
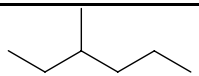
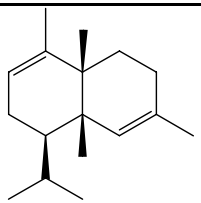
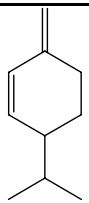
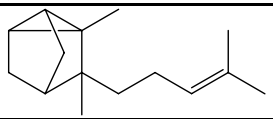
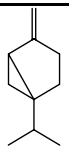
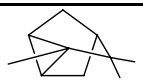
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
01.047	Longifolene		28	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.050	3-Methylhexane		0.061	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.052	alpha-Muurolene		0.24	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.055	beta-Phellandrene		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.056	alpha-Santalene		0.012	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: No adequate NOAEL	Additional data required		
01.060	1,1,7-Trimethyltricyclo[2.2.1.0.(2.6)]heptane		0.012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

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

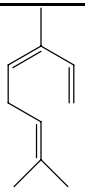
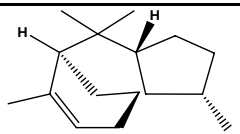
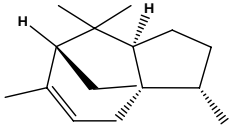
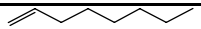
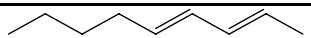
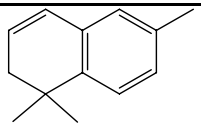
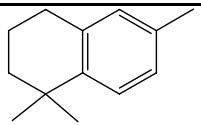
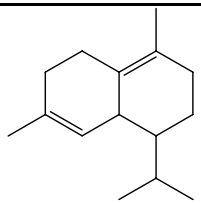
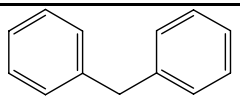
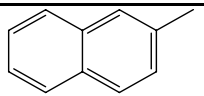
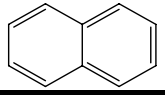
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
01.064	cis-3,7-Dimethyl-1,3,6-octatriene		14	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.066	2-Cedrene		0.97	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.067	8(14)-Cedrene		0.012	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		
01.078	2,4-Nonadiene		6.1	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.031	1,2-Dihydro-1,1,6-trimethylnaphthalene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.058	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.021	delta-Cadinene		0.15	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
01.036	Diphenylmethane		1.2	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.051	2-Methylnaphthalene		0.0012	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
01.053	Naphthalene		0.013	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

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

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

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

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

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

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

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

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

REFERENCES

Abe S and Sasaki M, 1977. Chromosome aberrations and sister-chromatid exchanges in chinese hamster cells exposed to various chemicals. J. Natl. Cancer Inst. 58(6), 1635-1641.

Anderson D and Styles JA, 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens. Appendix 2. The bacterial mutation test. Br. J. Cancer 37, 924-930.

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. Environ. Mol. Mutag. 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 Radic. Biol. Med, 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.

Brams A, Buchet JP, Crutzen-Fayt MC, DeMeester C, Lauwerys R and Leonard A, 1987. A comparative study, with 40 chemicals, of the efficiency of the salmonella assay and the SOS chromotest (kit procedure). Toxicol. Lett. 38, 123-133.

Brouns RE, Poot M, deVrind R, v Hoek-Kon T, Henderson PT and Kuyper CMA, 1979. Measurement of DNA-excision repair in suspensions of freshly isolated rat hepatocytes after exposure to some carcinogenic compounds. Mutat. Res. 64, 425-432.

CICAD, 1999. Concise International Chemical Assessment Document 6. Biphenyl. World Health Organization, Geneva.

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. Toxicol. Appl. Pharmacol. 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. Toxicol. Lett. 25, 33-40.

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

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, 1983a. Mutagenicity evaluation of beta-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 aactivity of environmetally important polycyclic aromatic hydrocarbons and their nitro derivates in the wing spot test of Drosophila melanogaster. Mutat. Res. 341, 235-247.

De Meester C, Mercier M and Poncelet F, 1981. Mutagenic activity of butadiene, hexachlorobutadiene, and isoprene. Ind. Environ. Xenobiotics, Proc. Int. Conference 27-30 May 1980, 195-203.

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

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

EC, 2000a. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

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

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

EFFA, 2005a. 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.

EFSA, 2004d. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 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). Adopted on 7 October 2004. EFSA-Q-2003-146.

EFSA, 2004e. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 7: Saturated and unsaturated aliphatic secondary alcohols, ketones, and esters of secondary alcohols and saturated linear or branched-chain coboxylic acids from chemical group 5 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 9 December 2004. EFSA-Q-2003-150.

EFSA, 2011k. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 25Rev2: Aliphatic and aromatic hydrocarbons from chemical group 31 (Commission Regulation (EC) No 1565/2000 of 18 July 2000).

EU-RAR, 2003. EUR 20763 EN European Union Risk Assessment Report. Naphthalene, vol. 33. Munn, S.J., Allanou, R., Berthault, F., de Bruijn, J., Musset, C., O'Conner, S., Pakalin, S., Pellegrini, G., Scheer, S., Vegro, S., (eds.). European Commission, European Chemicals Bureau, Institute for Health and Consumer Protection. Published by Office for Official Publications of the European Communities, Luxembourg.

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

FAO/WHO, 1967. Diphenyl. Food and agriculture organization of the united nations world health organization. FAO / WHO, 1967. FAO, PL: CP / 15; WHO / Food Add. / 67.32.

Flavour Industry, 2010a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-78rev1 [FL-no: 01.008].

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

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

Gocke E, 1999b. 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. Dissert. Abs. Int. 52, 694-B.

Gomes-Carneiro MR, Viana MES, Felzenszwalb I and Paumgartten FJR, 2005a. Evaluation of beta-myrcene, alpha-terpinene and (+)- and (-)-alpha-pinene in the salmonella/microsome assay. Food Chem. Toxicol. 43, 247-252.

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

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

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

Hirayama T, Nohara M, Shindo H and Fukui S, 1981. Mutagenicity assays of photochemical reaction products of biphenyl (BP) and O-Phenylphenol (OPP) with NO(x). Chemosphere 10(2), 223-228.

Houk VS, Schalkowsky S and Claxton LD, 1989. Development and validation of the spiral Salmonella assay: An automated approach to bacterial mutagenicity testing. Mutat. Res. 223, 49-64.

Hsia MTS, Kreamer BL and Dolara P, 1983. A rapid and simple method to quantitate chemically induced unscheduled DNA synthesis in freshly isolated rat hepatocytes facilitated by DNA retention of membrane filters. Mutat. Res. 122, 177-185.

IARC, 1999a. 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 alpha-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 (beta-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 Saftety and Health Law. Naphthalene. Carcinogenicity studies. Mutagenicity studies. Cited in CCRIS.

Jauhar PP, Henika PR, MacGregor JT, Wehr CM, Shelby MD, Murphy SA and Margolin BH, 1988. 1,3-Butadiene: induction of micronucleated erythrocytes in the peripheral blood of B6C3F1 mice exposed by inhalation for 13 weeks. Mutat. Res. 3-4, 171-176.

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

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

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

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

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

JECFA, 2005c. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.

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

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

JECFA, 2009c. Evaluation of certain food additives. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 952. Rome, 17-26 June 2008. http://whqlibdoc.who.int/trs/WHO_TRS_952_eng.pdf (May 2009)

JMPR, 1968. Joint Meeting of the FAO Working Party of Experts and the WHO Expert Committee on Pesticide Residues. 1967 evaluations of some pesticide residues in food. Diphenyl. Food and agriculture organization of the united nations world health organization. FAO / PL: 1967 / M / 11 / 1. WHO / Food Add. / 68.30. Rome, 4-11 December 1967.

Kaden, D.A., Hites, R.A., Thilly, W.G., 1979. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. Cancer Res. 39, 4152-4159.

Kauderer, B., Zamith, H., Paumgartten, J.R., Speit, G., 1991. Evaluation of the mutagenicity of beta-myrcene in mammalian cells in vitro. Environ. Mol. Mutagen. 18, 28-34.

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

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

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

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

Lankas GR, Baxter CS and Christian RT, 1978. Effect of alkane tumor-promoting agents on chemically induced mutagenesis in cultured V79 Chinese hamster cells. J. Toxicol. Environ. 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.

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. J. Health Sci. 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. Mutat. Res. 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. Proc. Nat. Acad. Sci. USA, 72(12), 5135-5139.

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. Environ. Mol. Mutag. 8(Suppl. 7), 1-119.

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

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. Environ. Mol. Mutag. 16 Suppl. 18, 138-167.

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.

Nohmi T, Miyata R, Yoshikawa K and Ishidate M, 1985. [Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests]. Bull. Natl. Inst. Hyg. Sci. (Eisei Shikenjo Hokoku) 103(60), 60-64. (In Japanese)

NTP, 1990e. 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, 1992g. 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, 1999d. 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, 2003c. Initial study results from a 90-day toxicity study on beta-myrcene in mice. Study number C99023.

NTP, 2004a. Draft study results of a 13-week gavage study in rats (beta-myrcene). Study no. C099023. http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm. Accessed 01/03/2005.

NTP, 2004b. Salmonella study results (beta-caryophyllene). Study no. 663295. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=87%2D44%2D5. Accessed 19/12/2007.

NTP, 2004c. Salmonella study results (beta-myrcene). Study no. A96914. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=123%2D35%2D3. Accessed 19/12/2007.

NTP, 2004d. Salmonella study results (cadinene). Study no. 385918. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction =ntpsearch.ntpstudiesforchemical&cas_no=29350%2D73%2D0. Accessed 19/12/2007.

NTP, 2004e. Salmonella study results (beta-cadinene). Study no. 519172. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=523%2D47%2D7. Accessed 19/12/2007.

NTP, 2004f. Mouse lymphoma study results (beta-cadinene). Study no. 138289. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=523%2D47%2D7. Accessed 19/12/2007.

NTP, 2004g. Sister chromatid exchange and chromosome aberration study results (beta-cadinene). Study no. 972780. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=523%2D47%2D7. Accessed 19/12/2007.

NTP, 2004h. Study results of micronucleus study in peripheral blood of mice (beta-myrcene). Study no. A06528. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=123%2D35%2D3. Accessed 19/12/2007.

NTP, 2004i. Salmonella study results (biphenyl). Study no. 512660. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=92%2D52%2D4. Accessed 19/12/2007.

NTP, 2004j. Salmonella study results (biphenyl). Study no. 773612. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=92%2D52%2D4. Accessed 19/12/2007.

NTP, 2004k. Salmonella study results (1-methylnaphthalene). Study no. 404676. [Online] http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=ntpsearch.ntpstudiesforchemical&cas_no=90%2D12%2D0. Accessed 19/12/2007.

NTP, 2010b. National Toxicology Program. Toxicology and carcinogenesis studies of beta-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/.

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

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 Cosmet. Toxicol. 7, 405-407.

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK and Neal SB, 1981. Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ. Mutag. 3, 11-32.

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. J. Toxicol. Environ. Health, Part B 6(2):161-183.

Rivedal E, Mikalsen SO, 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. Toxicol. In Vitro 14, 185-192.

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

Röscheisen C, Zamith H, Paumgartten F and Speit G, 1991. Influence of beta-myrcene on sister chromatid exchanges induced by mutagens in V79 and HTC cells. Mutat. Res. 264, 43-49.

Sakai M, Yoshida D and Mizusaki S, 1985. Mutagenicity of polycyclic aromatic hydrocarbons and quinines on *Salmonella typhimurium* TA97. Mutat. Res. 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. Mutat. Res. 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. Mutat. Res. 393(1-2), 23-35.

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

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. Mutat. Res. 517(1-2), 53-75.

Shelby MD, 1990. Results of NTP-sponsered mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. Environ. Health Perspect. 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. Mutat. Res. 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 Biol. Toxicol. 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. Biochem. Pharmacol. 46, 1529-1538.

Tummey AC, McKee RH and Przygoda RT, 1992. Evaluation of *in vivo* tumor promoters in a battery of *in vitro* assays. Environ. Mol. Mutag. 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

Wangenheim J and Bolcsfoldi G, 1988. Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis 3(3), 193-205.

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. Hum. Exp. Toxicol. 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 beta-myrcene in the *in vivo* cytogenetic bone marrow assay. Brazilian J. Med. Biol. Res. 26, 93-98.

Zeiger E, Anderson B, Haworth S, Lawlor T and Mortelmans K, 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutag. 19(21), 2-141.

ABBREVIATIONS

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